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In *Vitro* Root Induction and Acclimatization of Asian Pear (*Pyrus betulifolia)* in Field

Nyo Zin Hlaing[1]
Plant Tissue Culture Laboratory
Department of Biotechnology,
Ministry of Education, Kyaukse, Myanmar

Jiang Shuling[2]
Germplasm Resources and Breeding of Pear
Institute of Pomology (CAAS), Ministry of Agriculture,
Xingcheng, 125100, Liaoning, China

Abstract:- Explants of *P.betulifolia* were tested for micropropagation and rooting in vitro and acclimatization in vivo. In multiplication test, BA 0.5 mg/l combination was the best proliferation rate. Rooted shoots, root length and rooting percent were resulted the best in this data as 3.35, 2.33 cm and 67% respectively in ½ MS + 0.3 mg /l with dipping 10 second in 2 mg/l IBA (50% EtOH). But ½MS + 0.2 mg/l IBA with dipping 15s in the same IBA concentration provided the best number of roots (4.30). According to the rooting data, ½MS + 0.2 and 0.3 mg/l IBA were given roots but they were not good results as dipping method. Finally, the healthy survived 37.10% pear from in vitro rooted plants was grown in fields successfully.

Keywords:- *P.betulifolia, Acclimatization, ½MS, BA, IBA, EtOH.*

Abbreviation:
MS - Murashige and Skoog
BA - 6-Benzyladenine
IBA -Indole-3-butyric acid
IAA- Indole-3-acetic acid
Zt -Zeatin
GA3- Gibberellic acid

I. INTRODUCTION

Pears are cultivated in all moderate regions of the world. Pear belongs to the genus *Pyrus*, subfamily *Pomoideae* in the family *Rosaceae* and contains twenty two species. Pears are found many varieties of color, containing many changed of green, red, yellow or gold and brown etc. They are difficult to determine ripeness with their colors. There is different debate about the origin of pear from European Pear but the experts believed that European Pear (*Pyrus communis*) and Asian pear (*Pyrus betulifolia* and *Pyrus ussuriensis*) developed differently and the similar suitable period in story (uncertainly 1000 BC). But they are several other different between these two pears. Early in the 1500's, Eurpoean pioneers started to take pears to North America, where they were not clearly local or owned formerly. Nowadays, pear is grown and distributed around the world such as U.S, Argentina, Chile, China, South Korea and New Zealand. Among these countries, China is now the most grower of pear in the earth because they can produce about 15.5 million tons out of 21 million worldwide pear productions, almost three-quarters of the world total. The other 2.7 from Europe, 1.1 from Argentina and Chile, 0.8 from U.S and other least amount from New Zealand, Korea and other countries could respectively produce million tons of pears.

Asian pear, *Pyrus betulifolia* is a species in the genus *Pyrus* that contains approximately 36 to 124 species and also associates to the relative of the *Rosaceae* (Rose Family). This kind of pear is called Du Li in China and Birch-Leaf Pear in English. This species is pear tree native to East Asia and a deciduous wild pear tree of 10m height in North and Central China, and is often planted in gardens and parks for ornamental purposes, resistant to pear decline disease, limestone soil and drought condition. It is leafy forest type that kept from the prey of herbivores with stalks changed as frightening prickles. The flower are visited by bees and this fruit is nearly circle with width range between 5 and 12 mm, a greenish-brown outer cover with white dots and an elongated stem 3 to 4 times more remote than the fruit. Small pears mature at the end of August. The fresh of small pear is juicy.

Plant tissue culture techniques have quickly developed for plant micropropagation, plant cultivation, uniform planting material, disease free technique and for studying diifferent conditions of plant improvement in pear. Micropropagation is the rapid asexual in vitro multiplication of a desired plant. It is critical for both poliferation and stabilizing of an advanced series of horticultural crops, especially fruit plants. In pear, micropropagation was initiative succeeded in 1979 on pear rootstock OH x F 51 (Cheng, 1979) and scion variety Bartlett (Lane, 1979a). Since, the early data, major development has been taken in the various regions of in vitro pear multiplication. The present study of multiplication, rooting and acclimatization of *P. betulifolia* were described as below. The target of this research must be to establish ordinary rooting and acclimatization procedures and transfer of Asian pear explants to commercial production facilities.

II. MATERIALS AND METHODS

A. Plant Materials

The *in vitro* shoots of *Pyrus sps.* were applied as the plant sources for the adventage of this study. They were obtained from Institute of pomology, Chinese Academy of Agricultural Science, Xingcheng, Liaoning.

B. Multiplication for P.betulifolia

The shoots of *P.betulifolia* were in multiplication phase on MS medium added with 0.5 and 1.0 mg/l BA, 0.2

mg/l Zt, 01.IBA, 0.4mg/l IAA and 0.1 mg/l GA3 and M II that contained. Moreover, sucrose and agar also supplemented about 30 and 6 grams per liter respectively. Before autoclaving, the media of pH was balanced to 5.75 with 0.1 N KOH. The media were disinfected with autoclave (121˚C for 20 minutes). All cultures were placed to a growth room at 20 ± 3˚C with a light power of 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ brightened by white phosphorescent. They were taken subcultures every 4 weeks.

M I = 0.2 mg/l Zt + 0.5 mg/l BA + 0.4 mg/l IAA + 0.1 mg/l IBA + 0.1 mg/l GA3
M II = 0.2 mg/l Zt + 1.0 mg/l BA + 0.4 mg/l IAA + 0.1 mg/l IBA + 0.1 mg/l GA3

C. Root Formation of Asian pear

Firstly, about 3cm *P. betulifolia* shoots were taken from the above *in vitro* multiplication. They were grown on ½ MS basal medium added 0.2 and 0.3 mg/L of IBA (Indole-3-butyri acid). All the media were added 6 g/l agar and 15 g/l sugar respectively. The media were disinfected with autoclave (121˚C for 20 minutes). The explants were planted on this basal media under the sterilized laminar airflow. They were placed under darkness about 7 days and then transferred on light conditions about 7 days. After these conditions, some of plants were different seconds (10 and 15) quick dipped in 10mM or 2mg/L IBA dissolved in 50% Ethanol. Finally, shoots were planted on ½MS medium that was decreased to the fourth of salt concentrations without plant growth regulators (PGR) for two weeks. But without dipping IBA plants were not changed media until one month. All cultures were placed to a growth room at 20 ± 3˚C with a light power of 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ brightened by white phosphorescent. The below rooting counts were observed: rooting percentage, roots number, rooted shoots and the length of roots.

T I = ½ MS + 0.2 mg/l IBA
T I-1 = ½ MS + 0.2 mg/l IBA dipping IBA 10s
T I-2 = ½ MS + 0.2 mg/l IBA dipping IBA 15s
T II =½ MS + 0.3 mg/l IBA
T II-1 =½ MS + 0.3 mg/l IBA dipping IBA 10s
T II-2 =½ MS + 0.3 mg/l IBA dipping IBA 15s

D. Acclimatization

Explants were eliminated from the medium of culture bottles, rinsed alert with water without remaining medium, transferred to plastic pots containing sterile soil substratum and hardened on a chamber that provided 100% humidity for initial 2 weeks and gradually reduced the humidity up to 70-75%. The percent survival of plants was increased up to 39 %.

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 because of unequal numbers of shoot bottles in each treatment. Mean values for each treatment were calculated using Statistix 8.0. LSD all-pairwise comparisons test with Statistix 8.0 software to identify differences among treatments at a 95% level of confidence (P≤0.05) and results of multiple range tests was used to separate homogeneous groups among treatments. Graphs and multiplication test were analyzed with Microsoft Excel 2010.

III. RESULTS AND DISCUSSIONS

The results of this study were showed as below suitable head complemented with tables and graphs.

A. Multiplication of Pyrus betulifolia

Between the multiplication media of pear, M I was better multiplication rate than M II. The aim of this research was for rooting and acclimatization so this step result was shown only the multiplication of shoot rate. It was not counted the other results as the number of shoot and the length of shoot. But, in this result, M I and M II were significant different between them.

Tretments	Average Multiiplication	
M I	2.69 ± 1.24	2.64± 0.80
M II	2.48 ± 0.20	

Table 1:- Means and standard deviation of Multiplication on Asian pear (*P. betulifolia*)

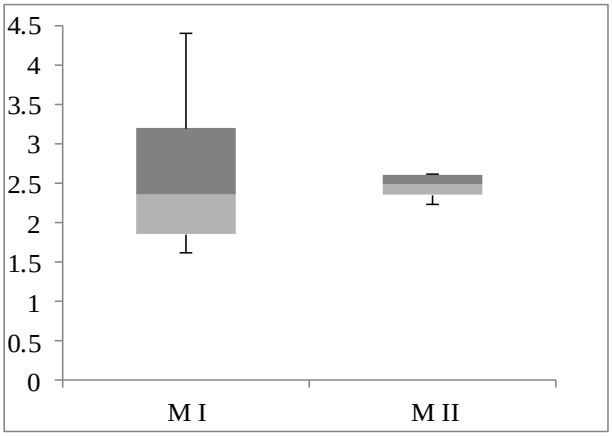

Fig 1:- The data summary for Multiplication of P.*betulifolia*

According to this result of multiplication of *P. betulifolia*, the more BA, the taller pear shoots but less multiplication rate was got. The previous researches were showed that proliferation increased BA level increased with either auxin. But in this research, increased BA level (1.0mg/l) was provided just the taller and stronger shoots. More new shoots were produced from BA 0.5 mg/l. It could be provided a lot of new shoots and stronger shoots for rooting stage. Other studies with pear have used only BA and no auxin for multiplicalionof *P. betufolia* (Dolcet-Sanjuan et.al., 1990; Nicolodi and Pieber, 1989). Dennis Y Yeo and Barbaram M. Reed (1995) observed that the best multiplication media for *P. betulifolia* "OPR 260" as Cheng media with 8µm BA and 0.5 IBA based on shoot multiplication and overall appearance, including leaf size, color and shoot height.

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B. In Vitro Root Induction of P.betufolia

Starting the initial root at the bottom of shoots was observed after two weeks. The rooted shoots, the number of roots, the percentage of root and the average number of roots were recorded after culture of the explants for four weeks under the established light and temperature conditions. Among the rooting media for *P.betuliolia*, T II-1 (IBA 0.3 and 10 second dipping IBA) was the best for three of root conditions. It was provided the best of rooted shoots (3.35), root length (2.33) and rooting percent (67.00%) respectively. But T I-2 (IBA 0.2 and 15 s dipping) was resulted for the best of number of roots (4.30) as shown in table II. This treatment was analyzed the second good for the other results. 0.5 s and 20 s dipping were tested for pear rooting but they were not given roots until one month later. Control was also provided the bad result of root so these results were not shown in this paper. The root length less than 0.5 cm was not counted in this data. They were no significant pairwise difference among the means.

Treatments	Rooted shoots	Number of Roots	Root Length (cm)	Rooting Percent (%)
T I	2.40	2.45	1.55	48.00
T I-1	3.24	4.07	1.85	64.75
T I-2	3.22	4.30	1.61	64.42
T II	3.10	2.74	1.55	62.00
T II-1	3.35	4.12	2.33	67.00
T II-2	2.35	3.12	1.33	47.00
CV(P≤0.05)	40.10	67.03	53.62	40.10

Table 2:- Effect of different IBA and different dipping time in IBA of mean of rooted shoots, roots number, the length of roots and rooting percent of *P. betulifolia*

Fig 2:- Root Induction of *P.betulifolia*

According to this data, IBA 0.3 mg/l was more suitable and early given for pear rooting. But in dipping time, 10 s was good for 0.3mg/l IBA as 15s of 0.2 mg/l. 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.

Removing light would protect auxin from degradation and reduce and reduces the activity of peroxidase which is an auxin degrading enzyme. This method must be more studied to be effective the species of dwarf pears. Drew observed that *in vitro* shooted roots were grown through a well hydrating gelled media that included exogenetic auxins and high containings of sucrose. So the agar-gelling medium was transparent; it transported light from the culture room that was recognized to delay rooting of plants. Crane and Hughes, 1990 researched that gas interchange was condensed and an oxygen shortage was occurred, but the amount of basal medium was needed a little and oxygen could not dispread till the roots. Moreover, the hardened roots were made in dark into the soil and good exposed things but nothing of sucrose and hormone, so roots were controlled by the shoots that got the requirements to cultivate. Therefore, 0.2 and 0.3 mg/l of IBA were resulted for pear rooting. High amount of auxin containing in rooting media was not needed as it decreased in rooting by making basal callus formation or by reducing the root elongation. Most studies were observed that *in vitro* rooting of *Pyrus spp;* such as apple and pear were needed auxins, especially IBA and IAA. IBA usage was found to play an essential role of these fruit trees rooting as they were different. IBA that is oxidized slowly and delayed degradation and slow movement may be the initial reason for well appearance of IBA as match up to other auxins.

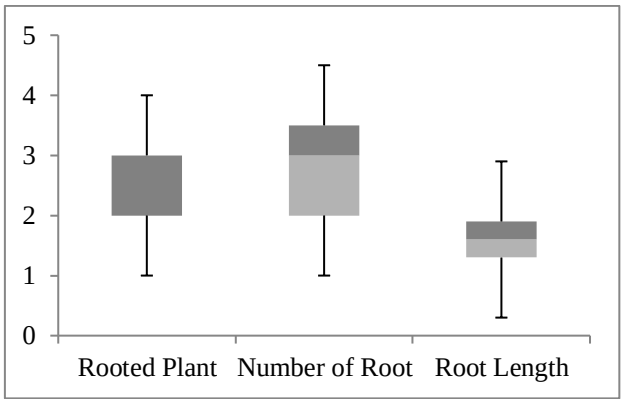

Fig 3:- The summary data of rooted plants, number of roots and root length of *P.betulifolia.*

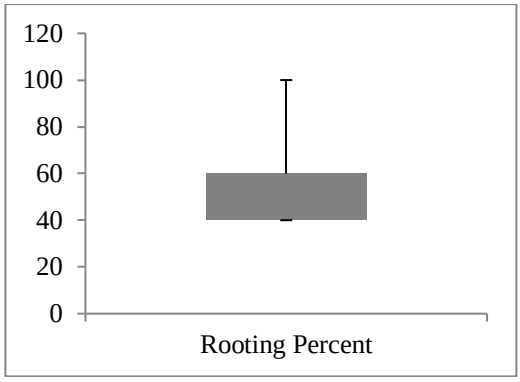

Fig 4:- The summary data of rooting percent of *P.betulifolia*

C. Acclimatization of P.betulifolia

The best rooting treatment was resulted 54.97% micropropagated shoots rooting. *In vitro* roots could be adapted to the soil after hardening condition. It can be supported to form new root tissues but they were different structures of the old roots. 39.74% in vitro rooted shoots survived after 2 weeks of acclimation in 100% relative humidity. After 2 weeks of acclimatization period, new leaves were added and roots become phenotypically strong and plants needed more water supplement to repair the loss of water by evaporation in terrestrial plant. And then, they were moved to the greenhouse until two months later.

Strain	Plants Transferred in Pot (%)	Surviving Plants (%)	Plants in Field (%)
P. betulifolia	54.97	39.74	37.10

Table 3:- Rooted plants established in Greenhouse about 3 months and transferred to the Fields in summer 2019.


Fig 5:- Hardening of *P.betulifolia* in Pots two months later and Planted in fields over 12 weeks.

Finally, 37.10% healthy pear plants must be grown successfully in field after 12 weeks later. Juan A. Marin; et al. observed that root hairs were adapted *in vitro* condition , but they were missed after removing from cultured bottle and would be regained soon as active root growth and a familiar soil materials.

IV. CONCLUSION

To conclude this paper, IBA 0.3mg/l was better for pear rooting but dipping method added was the best for root conditions. Dark condition held to induce root of pear in this research. The presence of low IBA, quick dip and darkness must be given roots of pear but should be studied more researches to confirm more getting strong roots of other species of pear, acclimate and produce commercial to the plants of different pear.

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