Plant Tissue Cult. & Biotech. **24**(1): 87-92, 2014 (June)

PTC&B

Induction of Direct Adventitious Shoot Regeneration in Pear (*Pyrus communis* L.)

Mahdieh Yousefiara*, Maryam Jafarkhani Kermani, Abdolreza Bagheri[1], Ali Akbar Habashi and Hamid Abdollahi[2]

Department of Tissue Culture and Gene Transformation, Agricultural Biotechnology Research Institute of Iran (ABRII), Mahdasht Road, P.O. Box 31535-1897, Karaj, Iran

Key words: Induction, Direct shoot regeneration, *Pyrus communis*, Thidiazuron

Abstract

Efficient direct shoot regeneration of two pear (*Pyrus communis* L.), namely *'bartlett'* and *'dargazi'* was successfully developed for the use in future genetic engineering research. The basal MS and NN media supplemented with different concentrations of TDZ (0, 2.5, 5, 7.5 μM) or BAP (0, 4, 8, 16 μM) in combination with NAA (1 μM) were compared. The result showed that direct adventitious shoot regeneration in pear was highly dependent on genotype, explant type and culture media. *'dargazi'* had higher rate of regeneration compared to *'bartlett'* and in both cultivars the highest per cent of regeneration was observed in lower sections of the leaves. Although the highest per cent of regeneration in *'bartlett'* (38) was attained in the NN medium containing 2.5 μM TDZ and 1 μM NAA, but the differences in shoot regeneration between media containing 5 or 7.5 μM TDZ and 1 μM NAA were not significant. The highest per cent of regeneration in *'dargazi'* (56) was obtained in NN medium containing 7.5 μm TDZ and 1 μm NAA.

Introduction

Pear is one of the oldest and the most important temperate fruit crops. It belongs to the genus *Pyrus*, the subfamily Maloideae (Pomoideae) of Rosaceae. Pear breeding by conventional methods is difficult and time consuming, because of its having high level of heterozigosity and the long juvenile period. Therefore, genetic improvement of pear through modern breeding, induced mutation and genetic engineering has been considered to be potential. *In vitro* direct regeneration is a general pre-requisite for these techniques. Various factors have

*Author for correspondence: <yousefiara@yahoo.com>. [1]Department of Biotechnology and Plant Breeding, Faculty of Agriculture , Ferdowsi University of Mashhad, P. O. Box 91775-1163, Mashhad, Iran. [2]Department of Horticultural Research, Seed and Plant Improvement Institute, P.O. Box 4119, Karaj, Iran.

been examined for pear regeneration, including; the type and orientation of explants (Caboni et al. 2002, Lane et al. 1998), plant growth regulator combinations and basal salt composition (Caboni et al. 1999, Abdollahi et al. 2006, Tang et al. 2008), gelling agents (Chevreau et al. 1997), darkness (Leblay et al. 1991, Liu et al. 2009), carbohydrates (Chevreau et al. 1989, Leblay et al. 1991) and some additives like antibiotics (Predieri et al. 1989, Caboni et al. 1999) or silver nitrate (Liu et al. 2009). Furthermore, some reports have shown that type of cytokinin in the shoot proliferation medium can affect shoot regeneration from *in vitro* explants (Bell et al. 2009).

The aim of the present investigation was to develop an efficient protocol to regenerate adventitious shoots from leaf explants of two commercial pear cultivars (*'bartlett'* and *'dargazi'*), in order to use the technique in further genetic engineering research.

Materials and Methods

In vitro shoots of two pear (*Pyrus communis* L.) cultivars (*'bartlett'* and *'dargazi'*) were supplied by Agricultural Biotechnology Research Institute of Iran (ABRII). The nodal explants were proliferated in modified QL medium (Quoirin and Lepoivre 1977) containing 1 μM NAA, 1 μM BA and 2 μM 2ip, 30 g/l sucrose and 7 g/l agar. The leaves from *in vitro* grown plants were used in the regeneration experiments. The pH of all media was adjusted to 5.8 before adding agar and autoclaved for 15 min at 121°C and 1.2 kPa. Petri dishes containing explants were incubated at 16 hrs photoperiod under cool-white fluorescent light at a PPFD of 60 μmol/m^2/s, at 22 ± 2°C.

Leaves from *in vitro* grown plants were cut perpendicular to the midrib into three sections; lower (with petiole), middle and upper and each considered as explants. The explants were placed on the culture media with the adaxial side on the media in 7 mm Petri dishes. MS or NN (Nitsch and Nitsch 1969) supplemented with different combinations of TDZ or BAP and NAA were used. Sixteen treatments including four concentrations of BAP (0, 4, 8 and 16 μM) which were defined as B0, B1, B2 and B3. Four concentrations of TDZ (0, 2.5, 5 and 7.5 μM) which were defined as T0, T1, T2 and T3 were used in combination with NAA (1 μM). The cultures were kept in the dark for four weeks, then they were subcultured in the same media composition and transferred to the light condition. After eight weeks, per cent of regenerated shoots were recorded.

The experiments were designed in a factorial based completely random design. Three replicates with 5 explants in each were used in each treatment. The data from all experiments were statistically analyzed using MSTAT-C and SAS. Mean values were evaluated at a $p < 0.01$ level of significance using DMRT.

Results and Discussion

Adventitious shoots developed on the NN medium containing various concentrations of TDZ or BAP. However, explants in all the treatments in MS did not generate adventitious shoots. Effectiveness of NN medium for direct regeneration of pear has also been reported by Sun et al. (1998) who stated that NN culture medium was more suitable than MS. The main differences among MS and NN media are in ionic concentration of ammonium, nitrate and their total ionic concentration. Leblay et al. (1991) reported that ammonium/nitrate ratio of 1 : 3 are essential in direct shoot regeneration of pear. Tang et al. (2008) examined six ratios including 1 for ammonium and 2, 3, 4, 5 and 7 for nitrate. They suggested that at ammonium/nitrate ratio (1 : 7) 97 per cent regeneration was achieved. Moreover, the NN media contains different types of vitamins and also higher amounts of nicotinic acid compared to MS. Other investigations have shown that decreasing the concentration of macro elements (using half strength MS) could have positive effect on regeneration (Chevreau et al. 1989, Leblay et al. 1991, Liu et al. 2009).

In both *'dargazi'* and *'bartlett'* the highest rates of regeneration (28 and 12%, respectively) were achieved when lower section of leaves was used (Fig. 1). Tang et al. (2008) also reported that in different cultivars maximum regeneration was achieved from basal leaf explants possessing petioles. The difference in regeneration ability of explants might be due to differences in the levels of endogenous hormones or an interaction between the endogenous and exogenous

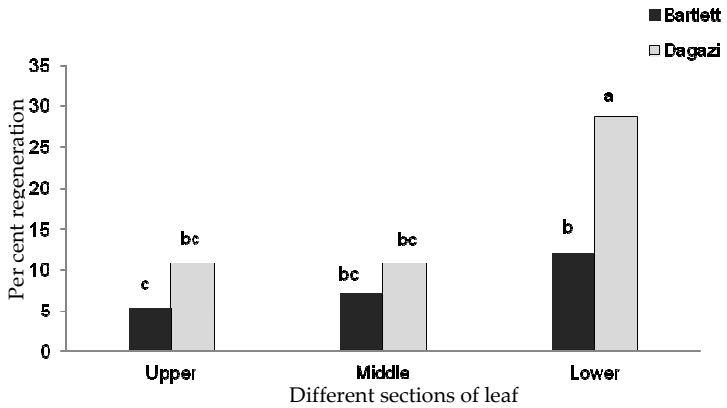


Fig. 1. Effect of explant type on adventitious shoot regeneration of pear. Means in each column with different letters show significant differences according to DMRT ($p \leq 0.05$).

hormone (Tang et al. 2008). Furthermore, the need of different leaf sections to hormonal concentration varied for different regeneration purposes. Tang et al. (2000) reported that higher concentrations of hormones were needed for

organogenesis from distal and medium parts of *Prunus cerasus* cotyledons than those for proximal parts.

In all the media containing BAP or TDZ shoots regenerated from explants (Fig. 3a) whereas in the media without cytokinins only roots developed on the explants (Fig. 3b). The highest per cent (56) of regeneration in *'dargazi'* was obtained in NN medium containing 7.5 μM TDZ and 1 μM NAA (Fig. 2).

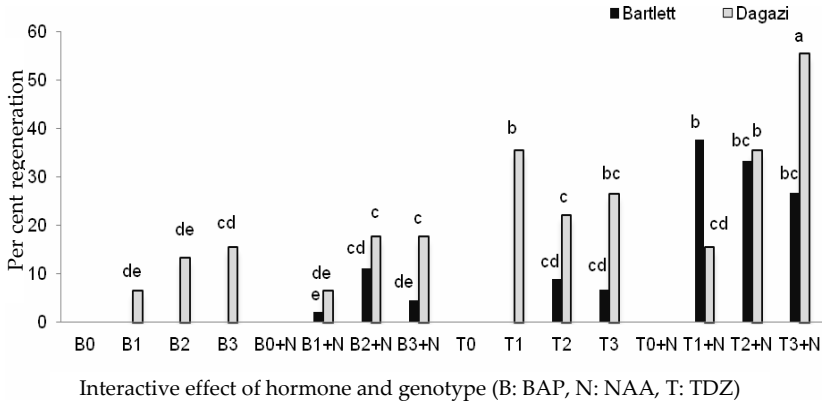
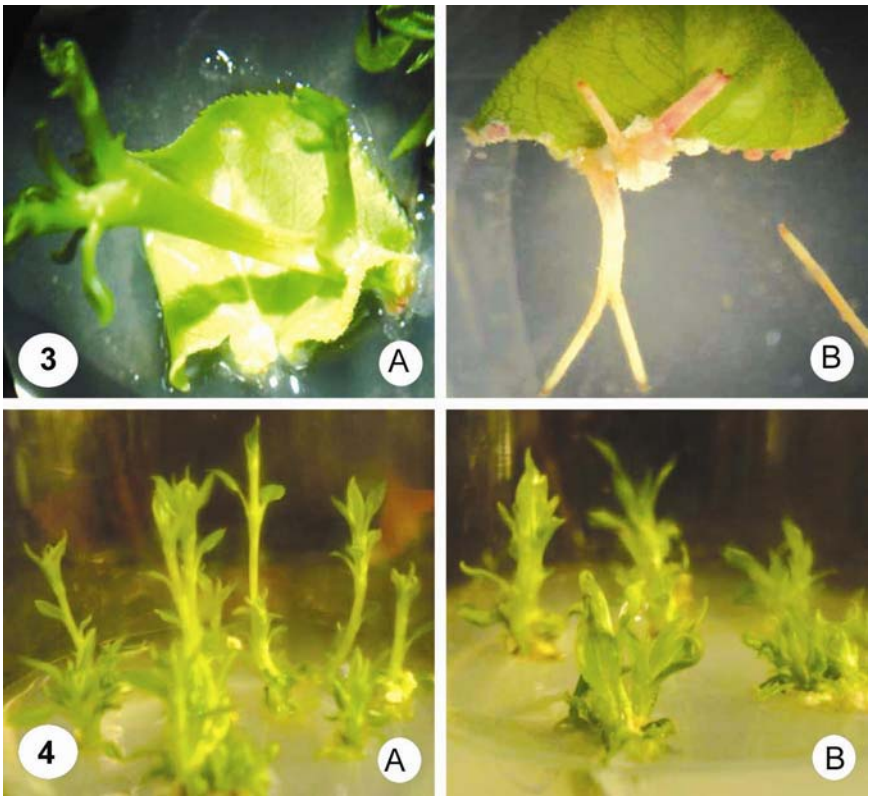


Fig. 2. Interactive effect of hormone on adventitious shoot regeneration of pear. Means in each column with different letters show significant differences according to DMRT ($p \leq 0.05$).

Although the highest per cent (38) of regeneration in *'bartlett'* was attained in the NN medium containing 2.5 μM TDZ and 1 μM NAA, but the differences in shoot regeneration between this medium and the NN media containing 5 or 7.5 μM TDZ and 1 μM NAA were not significant (Fig. 2). In both cultivars regeneration rates were significantly lower in NN media containing BAP compared to the media containing TDZ (Fig. 2). However, the regenerated shoots from BAP treatments had normal shape, whereas, shoots derived from TDZ treatments were short, intensive and with small leaves (Fig. 4).

Several reports have demonstrated the positive effects of TDZ on pear regeneration. Chevreau et al. (1989) reported that TDZ was more effective than BAP. Leblay et al. (1999) investigated different concentrations of TDZ (up to 48 μM), and concluded that TDZ concentrations more than 12 μM were preventive for pear regeneration. Liu et al. (2009) demonstrated that using cytokinin in combination with auxin promote pear regeneration. They showed that combination of TDZ with IBA was more effective than combination of TDZ with NAA. Caboni et al. (1999) reported that NAA had positive effect whereas IBA was ineffective in pear regeneration.

Effective role of genotype on pear regeneration and different organogenesis capacity in pear genus has been reported by many authors (Chevreau et al. 1989, Lane et al. 1998, Caboni et al. 2002).



Figs 3-4. 3A. Direct shoot regeneration from explant in medium containing cytokinin. 3B. Direct root regeneration from explant in medium lacking cytokinin. 4A. Normal shoots growing from adventitious shoots originated on the medium containing BAP. 4B. Compact and short shoots growing from adventitious shoots originated on the medium containing TDZ.

Present investigation demonstrated that direct adventitious shoot regeneration in pear was highly dependent on genotype and explant type and culture media were also effective in the regeneration capacity. The maximum per cent of regeneration was observed in lower sections of the leaves of *'dargazi'* in NN medium containing 7.5 µm TDZ and 1 µm NAA.

Acknowledgements

This work was funded by Agricultural Biotechnology Research Institute of Iran (Project number: 12-05-05-8602-87002).

References

Abdollahi H, Muleo R and **Rugini E** (2006) Optimisation of regeneration and maintenance of morphogenic callus in pear (*Pyrus communis* L.) by simple and double regeneration techniques. Scientia Horticulturae **108**: 352-358.

Bell RL, Srinivasan C and **Lomberk D** (2009) Effect of nutrient media on axillary shoot proliferation and preconditioning for adventitious shoot regeneration of pears. In Vitro Cellular and Developmental Biology-Plant **45**: 708-714.

Caboni E, D'Angeli S, Chiappetta A, Innocenti AM, Van Onckelen H and **Damiano C** (2002) Adventitious shoot regeneration from vegetative shoot apices in pear and putative role of cytokinin accumulation in the morphogenetic process. Plant Cell, Tiss. and Org. Cult. **70**: 199-206.

Caboni E, Tonelli MG, Lauri P, D'Angeli S and **Damiano C** (1999) *In vitro* shoot regeneration from leaves of wild pear. Plant Cell, Tiss. and Org. Cult. **59**: 1-7.

Chevreau E, Skirvin RM, Abu-Qaoud HA, Korbin SS and **Sullivan JG** (1989) Adventitious shoot regeneration from leaf tissue of three pear (*Pyrus* sp.) cultivars *in vitro*. Plant Cell Reports **7**: 688-691.

Chevreau E, Mourgues F, Neveu M and **Chevalier M** (1997) Effect of gelling agents and antibiotics on adventitious bud regeneration from *in vitro* leaves of pear. In Vitro Cellular and Developmental Biology-Plant **33**: 173-179.

Lane WD, Iketani H and **Hayashi T** (1998) Shoot regeneration from cultured leaves of Japanese pear (*Pyrus pyrifolia*). Plant Cell, Tiss. and Org. Cult. **54**(1): 9-14.

Leblay C, Chevreau E and **Raboin LM** (1991) Adventitious shoot regeneration from in vitro leaves of several pear cultivars (*Pyrus communs* L.). Plant Cell, Tiss. and Org. Cult. **25**(2): 99-105.

Liu J, Zhang X, Poudyal BK and **Zhang Y** (2009) Adventitious shoot regeneration from grown 'Zhongli 1' pear (*Pyrus* spp.). Frontiers of Agriculture in China **3**(1): 60-66.

Nitsch JP and **Nitsch C** (1969) Haploid plants from pollen grains. Science **163**: 85-87.

Predieri S, Fasolo FMF, Passey AJ, Ridout MS and **James DJ** (1989) Regeneration from *in vitro* leaves of Conference and other pear cultivars (*Pyrus communis*). J. Horti. Sci. **64**: 553-559.

Quoirin M and **Lepoivre P** (1977). Improved medium for *in vitro* culture of *Prunus* spp. Acta Horticulturae **78**: 437-442.

Sun QR, Sun HY and **Zheng HJ** (1998) The promotion of ethylene inhibitor $AgNO_3$ on buds regeneration from pear leaves (*Pyrus* species) grown *in vitro.* Deciduous fruits **4**: 1-2 (in Chinese).

Tang HR, Ren ZL and **Krczal G** (2000) Somatic embryogenesis and organogenesis from immature embryo cotyledons of three sour cherry cultivars (*Prunus cerasus* L.*).* Scientia Horti. **83**: 109-126.

Tang H, Luo Y and **Liu C** (2008) Plant regeneration from *in vitro* leaves of four commercial *Pyrus* species. Plant, Soil and Environ. **54**(4): 140-148.