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In vitro rooting of *Pyrus glabra* Boiss. microshoots

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Abstract
Fars province is one of the natural habitats of *Pyrus* species in Iran. Harvesting of fruits, deforestation, expansion of agriculture and overgrazing in recent years has threatened the wild pear open forests and imposed detrimental effects on their natural regeneration, therefore, investigation on propagation of wild pear species for afforestation is needed**.** This research was carried out to develop a suitable procedure for rooting of *Pyrus glabra* Boiss. *in vitro* propagated shoots. The shoots were obtained by culturing nodal segments or shoot tips of *P. glabra* on DKW medium containing 1.0 mgl^{-1} BA and 0.01 mgl^{-1} IBA. The best *in vitro* rooting procedure consisted of two phases: *in vitro* root induction and *ex vitro* root development. For root induction *P. glabra* shoots were cultured on MS medium (half strength macronutrients) containing 50 mgl^{-1} IBA and incubated in the darkness for 24 hours. For root development, shoots were transferred from root induction medium, washed with tap water, planted in moistened Jiffy-7 pots and placed in a propagator with 90-95% relative humidity under lighted conditions. Using this method, 100 percentages of shoots rooted and plantlets transferred to greenhouse successfully.

Keywords: Root induction; Root development; IBA; Wild pear

Introduction
Pyrus with about 22 species is one of the most important genera of Rosaceae (Callice and Westwood, 1973). Iran, with more than 10 species, is one of the important genetic resources of *Pyrus* in the world (Schönbeck-Temesy, 1975). He and khatamsaz (1992) reported that 12 species of *Pyrus*, including *Pyrus glabra* Boiss. and *Pyrus syriaca* Boiss. grow on the Iranian plateau. Khatamsaz (1992) reported that *Pyrus glabra* Boiss. and *Pyrus syriaca* Boiss. grow in the open forest of Dehkohne, Sepidan county, Fars province, Iran. Although the province is one of the native habitats of *Pyrus*, however, there is scarce information about the propagation of these species. The area of wild pear open forests in the Fars province is about 35000 ha, of which 30000 ha is located in Sepidan county, 100 km North of Shiraz (Hamzepour and Bordbar, 1999). As it grows wild in a wide range of soil types, the species are considered to be tolerant to drought, salinity, pests and diseases. Therefore, wild pear species (especially *P. glabra*) could be potentially used as a rootstock for common pear (*Pyrus communis*). Harvesting of fruits, deforestation, expansion of agriculture and overgrazing in recent years has threatened the wild pear open forests and imposed detrimental effects on their natural regeneration. This could lead to the loss of this valuable *Pyrus* genetic resource, therefore, investigation on propagation of wild pear species for afforestation and as a rootstock for common pear is needed**.** Akbari Mousavi and Saadat (2006) reported that 60 days stratification was the best treatment for removing dormancy in *P. glabra* seeds. Pear is a cross pollinated plant and the high genetic variability of seeds may lead to excessive variation affecting subsequent performance as rootstocks. It would be desirable, therefore, to develop methods of vegetative propagation of *P. glabra* such that sufficient quantities of rootstock with uniform characteristics could be produced. Pear cultivars have proven to be difficult to root from hardwood or softwood cuttings (Fadi and Hartmann, 1967). Shibly *et al.* (1997) reported that rooting in wild pear cuttings over 1 year of trials was unsuccessful. In addition, asexual propagation by conventional methods does not allow original clones to be

obtained in industrial quantities. In this respect, *in vitro* techniques would seem to be the most practical way to obtain rooted clonal material.

Two basic methods have been used for rooting of *Pyrus in vitro* produced shoots, a low concentration of auxin in semi-solid medium and a quick dip in a high concentration of auxin followed by transferring to medium free of growth regulators for root development (Barros *et al*., 2005). Thakur and Kanwar (2008) reported that rooting percentage of *P. pyrifolia* microshoots was significantly higher on solid medium compared to liquid medium irrespective of the concentration of used growth regulators. The rooting response on media containing NAA was better in comparison with IBA at lower concentrations (0.125 and 0.25 mgl^{-1}). On the contrary, at higher auxin concentrations (0.5-2.0 mg l^{-1}) significant higher rooting was observed with IBA compared to NAA, though the rooting response was poor. A combination of NAA and IBA at lower concentrations (0.125 and 0.25 mg l^{-1}) resulted in significant improvement of rooting percentage in comparison to NAA or IBA alone. The highest rooting percentage (81.47) was obtained on solid medium with a combination of 0.25 mgl^{-1} NAA and 0.25 mgl^{-1} IBA. A higher number of roots per microshoots were obtained on solid medium than on liquid medium. The number of roots per shoot was higher on media containing 0.125-0.250 mg l^{-1} NAA in comparison with those containing the same concentration of IBA. The root length in solid medium was significantly longer than that on liquid medium (Thakur and Kanwar, 2008). Shibly *et al.* (1997) reported that IBA, IAA and NAA induced *in vitro* rooting of *P. syriaca* and a maximum of 72% rooting was achieved at 3.0 $mg1^{-1}$ IAA. NAA has been reported to be more favorable auxin for pear rooting (Viseur, 1987; Dolcet-Sanjuan *et al.,* 1990; Al-Maani *et al.,* 1994). The best rooting in pear cultivars 'Passe Crassane' and 'Williams' was reported when using 0.2 mg 1^{-1} NAA (Al-Maani *et al.,* 1994). Rossi *et al.* (1991) reported 75% rooting in *P. cczlleryuna* D6**.** after initial darkness for 3 days and NAA (0.5-l.0 mg 1^{-1}).

In vitro rooting of *P. glabra* microshoots has not so far been reported. This paper reports rooting of *in vitro* propagated shoots of *P. glabra* for the first time.

Materials and methods

Shoot tips or nodal segments originated from selected trees of *P. glabra* open forests in Dehkohne region, Sepidan county, Fars province were used as explants for shoot multiplication. Shoots used for rooting experiments were obtained by culturing explants on DKW (McGranahan *et al*.,1987) medium solidified with 2.2 gl^{-1} Phytagel containing 30 gl^{-1} sucrose, 1.0 mgl^{-1} BA and 0.01 mgl^{-1} IBA (Saadat *et al.*, 2012). The shoots used were 3 to 5 cm long with an average of 6-8 nodes and the basal parts of shoots were disbudded for 5-10 mm before culturing on rooting media.

The effects of different concentrations of IBA on in vitro rooting of P. glabra shoots

This experiment was carried out to study the effects of different concentrations of IBA on *in vitro* rooting of *P. glabra* shoots. Murashige and Skoog (1962) medium (MS medium) containing 30 gl^{-1} sucrose and solidified with 8.0 gl^{-1} Difco Bacto agar was used as basal medium. The experiment was laid out as a completely randomized design with five levels of IBA (0.01, 0.1, 0.2, 0.5 and 1.0 mgl^{-1}) as treatments. Each treatment consisted of five replications, each replication containing 4 shoots. The cultures were incubated in a growth chamber with a light intensity of 45 µmol $m^{-2}s^{-1}$, with a 16-hour photoperiod at 27±1°C. Rooting indices including rooting percentage, number of primary roots per rooted shoot and length of roots were collected after 4 weeks.

The effects of different auxins and duration of shoot incubation on root induction medium on rooting performance of P. glabra shoots

This experiment was carried out in two phases: (A) root induction and (B) root development. Root induction of shoots was carried out on MS medium (half strength macronutrients) containing 30 gl^{-1} sucrose, 50 mg l^{-1} IBA or NAA and solidified with 9.0 gl^{-1} Difco Bacto agar. During the root induction period, the cultures were incubated in an incubator at 27±1°C in the darkness. The experiment was laid out as a factorial experiment with two different kinds of auxins, IBA or NAA as factor A and three different incubation period (30, 40 and 50 hours) of shoots on root induction media. For root development, the shoots were

transferred to MS medium free of growth regulators with 30 gl^{-1} sucrose and solidified with 9.0 gl^{-1} Difco Bacto agar. During the root development phase, the cultures were incubated in a growth chamber with a light intensity of 45 µmol m^{-2} s^{-1} and a 16-hour photoperiod at 27±1°C.

Treatments arranged in a completely randomized design with five replications, each replication containing four shoots. Data were collected after 5 weeks on root development medium.

The effects of different rooting treatments on rooting performance of P. glabra shoots

This experiment was carried out in two phases: (A) root induction and (B) root development. Root induction of shoots was carried out on MS medium (half strength macronutrients) containing 30 gl^{-1} sucrose, 50 mg l^{-1} IBA and solidified with 9.0 gl^{-1} Difco Bacto agar. During the root induction period, the cultures were incubated in an incubator at 27±1°C in the darkness. The experiment was laid out as a completely randomized design with five treatments including: 1. Incubation of shoots on induction medium for 12 hours. 2. Incubation of shoots on induction medium for 24 hours. 3. Incubation of shoots on induction medium for 48 hours. 4. Incubation of shoots on induction medium for 72 hours. 5. Incubation of shoots on induction medium for 24 hours and transfer to jiffy-7 pots for development of roots. For root development, shoots that were cultured on root induction media in treatments 1, 2, 3 and 4 were transferred to MS medium free of growth regulators. Each treatment consisted of five replications, each replication containing four shoots. Data were collected after 5 weeks on root development medium.

All statistical analyses were performed using the programme GLM on SAS (SAS Institute, 1988). Mean comparisons were performed by Duncan's multiple range test.

Results

The effects of different concentrations of IBA on in vitro rooting of P. glabra shoots

Based on the results obtained in this experiment, there was no significant difference for rooting indices among all shoots cultured on media containing different concentrations of IBA after one month being in culture (Table 1).

Table 1- The effects of different IBA concentrations on rooting performance of *P. glabra* shoots after five weeks*.

IBA concentrations	Rooting percentage	Average number of primary roots	Average length of primary roots (cm)
0.01 mg l^{-1}	40.0 a	2.64 a	1.21 a
0.1 mg l^{-1}	48.0 a	3.92 a	2.79 a
0.2 mg l^{-1}	24.0 a	5.67 a	1.45 a
0.5 mg l^{-1}	40.0 a	5.58 a	1.68 a
1.0 mg l^{-1}	12.0 a	3.00 a	1.27 a

* Values followed by the same letter in each column are not significantly different (P<0.01).

The effects of different auxins and duration of shoot incubation on root induction medium on rooting performance of P. glabra shoots

The rooting percentage of shoots cultured on root induction medium containing 50.0 mgl^{-1} IBA was not significantly different from those cultured on media containing 50.0 mgl^{-1} NAA for rooting percentage and average number of primary roots (Table 2). Average length of primary roots per rooted shoots cultured on root induction medium containing 50.0 mgl^{-1} IBA was significantly longer than those cultured on media containing 50.0 mgl^{-1} NAA.

Table 2- The effects of different auxins and duration of shoot incubation on root induction medium on rooting performance of *P. glabra* shoots.

Auxin	Rooting percentage	Average number of primary roots	Average length of primary roots (cm)
IBA	53.85 a	7.21 a	3.09 a
NAA	50.0 a	5.61 a	1.92 b
Duration of shoot incubation			
30 hours	40.0 a	7.81 a	2.62 a
40 hours	47.5 ab	5.06 a	1.88 a
50 hours	71.9 b	6.34 a	3.02 a
Interaction	ns	ns	ns

* Values followed by the same letter in each column are not significantly different (P<0.05).

Maximum rooting percentage was observed by shoots cultured on root induction medium for 50 hours and significantly more than those shoots cultured on root induction medium for 30 hours. No significant difference was observed for rooting percentage between shoots cultured on root induction medium for 40 hours and 50 hours. There was no significant difference among shoots cultured on root induction medium for different duration times for average number and average length of primary roots (Table 2). The interaction of different kinds of auxins and different induction durations of cultures on root induction medium was not significant for all rooting indices.

The effects of different rooting treatments on rooting performance of P. glabra shoots

All shoots cultured on root induction medium containing 50 mg l^{-1} IBA for 24 hours and transferred to moistened Jiffy-7 pots rooted and significantly higher than those shoots which were cultured on root induction medium for 12, 24 and 48 hours and transferred to MS medium free of growth regulators for root development (Table 3). No significant difference was observed for rooting percentage of shoots cultured on root induction medium containing 50 mg l^{-1} IBA for 24 hours and transferred to moistened Jiffy-7 pots and shoots cultured on root induction medium for 72 hours and transferred to MS medium free of growth regulators for root development. Rooting percentage of shoots cultured on root induction medium for 72 hours was significantly greater than those shoots cultured on root induction medium containing 50 mg l^{-1} IBA for 12 hours.

Table 3- The effects of different rooting treatments on rooting performance of *P. glabra* shoots five weeks after transfer to root development media*.

Rooting treatments		**Rooting percentage**	**Average number of primary roots**	**Average Number of secondary roots**	**Average length of primary root (cm)**
Duration of shoot incubation on root induction medium	**Root development medium**				
12 hours	MS medium free of growth regulators	25.0 a	2.13 a	10.83 a	6.33 a
24 hours	MS medium free of growth regulators	54.2 ab	4.17 a	6.47 a	3.07 b
48 hours	MS medium free of growth regulators	53.3 ab	6.25 a	9.42 a	3.98 ab
72 hours	MS medium free of growth regulators	85.0 bc	14.52 b	4.88 a	3.86 ab
24 hours	Jiffy-7 pots	100.0 c	6.10 a	11.2 a	.12 b

*Values followed by the same letter are not significantly different (P<0.01).

The average numbers of primary roots of shoots were cultured on root induction medium containing 50 mg l^{-1} IBA for 72 hours was significantly higher than those shoots

which were cultured on other rooting treatments. Average length of primary roots of shoots were cultured on root induction medium for 12 hours was significantly higher than those shoots which were cultured on root induction medium for 24 hours and transferred to MS medium free of growth regulators or Jiffy-7 pots for root development (Table 3). No significant difference was observed among shoots cultured on all rooting treatments for average number of secondary roots. Callus formation at the basal ends of shoots which were transferred to Jiffy-7 pots for root development was least and the quality of plantlets was better than those plantlets produced on MS medium free of growth regulators.

Discussion

For rooting of *in vitro* propagated *P. glabra* shoots, two different procedures were used. The first one was the culture of shoots on a medium containing low concentration of auxin for root induction and development on the same medium. Utilization of this procedure resulted in a maximum of 48 percent rooting at 0.1 mgl^{-1} IBA. This method is not an optimized procedure for commercial micropropagation of *P. glabra*. Shibly *et al.* (1997) reported that a maximum of 72% rooting of *P. syriaca* was achieved at 3.0 $mg1^{-1}$ IAA.

The second procedure was rooting of *in vitro* propagated *P. glabra* shoots in two separate successive phases: *in vitro* root induction at high concentration of auxin for a short period of time and *ex vitro* root development in nutrient media free of plant growth regulators or jiffy-7 pots. This method is the best procedure for rooting of *in vitro* propagated *P. glabra* shoots and in agreement with Barros *et al.* (2005) who reported a two phase *in vitro* rooting procedure for Portuguese pear cultivars (*Pyrus communis*). This is the first report of two phase rooting of wild pear species. For *in vitro* root induction the most efficient treatment was incubation of shoots on MS medium (half strength macronutrients) containing 50 mgl^{-1} IBA under dark conditions for 24 hours. For root development, shoots were planted in moistened Jiffy-7 pots and placed in a propagator with 90-95% relative humidity in lighted conditions. High improvement of rooting (100% rooting) was obtained by the utilization of Jiffy-7 pots for root development (Table 3). This procedure was suggested by Saadat and Hennerty (1999) for *in vitro* rooting of Persian walnut microshoots.

No significant difference was obtained among media containing 50 mgl^{-1} IBA or NAA for rooting percentage and average primary roots per shoot, but average length of roots was significantly longer on media containing IBA. IBA is the preferred auxin for many plants and most of researcher incorporated IBA into media to induce rooting. Selection of the most appropriate auxin depends not only on its ability to induce rooting, but also on the quality of produced roots. Alderson *et al.* (1987) reported that NAA at 0.5 mgl^{-1} or NAA plus IBA (0.5-1.0 mgl^{-1} of each) give rise to swollen roots and callusing on *Prunus tenella* shoots while IBA at 0.5 mgl^{-1} produced normal thin roots and little callus in agreement with our results.

The utilization of Jiffy-7 pots for root development of *P. glabra* shoots is very easy with the following advantages. Jiffy-7 pots contain all the necessary nutrients to secure healthy plant growth. The use of the Jiffy-7 pots eliminated an expensive, labour intensive transfer of *in vitro* root-induced shoots to the *in vitro* root development medium and could be considered as a time and money saving treatment for commercial mass propagation of *P. glabra*. In addition, they allowed the generation of normal thin, flexible white primary roots with secondary roots, in comparison to thick, brittle, usually dark primary roots without secondary roots in gelified medium. *Ex vitro* developed leaves could also photosynthesize and together with the well-developed root system, gave good survival of plantlets after transfer to the greenhouse. In addition, the use of Jiffy-7 pots eliminated an expensive, labour intensive transfer of *in vitro* root-induced shoots to the *in vitro* root development medium and could be considered as a time and money saving treatment for commercial mass propagation of *P. glabra*. As well as, the storage and transportation of plantlets in Jiffy-7 pots are possible.

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