

# Micropropagation of Three *Pyrus* Rootstocks

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**Abstract.** Explants of three rootstock selections *Pyrus calleryana* Dcne 'Oregon Pear Rootstock (OPR) 157', *P. betulifolia* Bunge 'OPR 260', and *P. communis* L. 'Old Home' x 'Farmingdale 230' ('OH x F 230') were initiated from forced branches of field-grown trees. 'OPR 260' and 'OH x F 230' shoots cultured on Cheng medium with IBA proliferated better than those on NAA. NAA and IBA at concentrations >0.5  $\mu\text{M}$  inhibited shoot multiplication. Overall, the best micropropagation medium for 'OPR 260' and 'OH x F 230' was Cheng medium with 8  $\mu\text{M}$  BA and 0.5  $\mu\text{M}$  IBA. Shoot multiplication of 'OPR 157' was best on 8  $\mu\text{M}$  BA and better on low NAA (0.5  $\mu\text{M}$ ) or no auxin than on IBA. 'OH x F 230' rooted easily (>80%) with all IBA and NAA treatments. The best rooting treatment (42.9%) for 'OPR 260' was 10  $\mu\text{M}$  IBA in darkness for 1 week; for 'OPR 157' (23.9%), it was a 15-second dip in 10 mM NAA. Only rooted plantlets survived 4 weeks of greenhouse acclimatization. Chemical names used: N<sup>6</sup>-benzyladenine (BA); indole-3-butyric acid (IBA); naphthaleneacetic acid (NAA).

Rootstocks are used to control or improve the growth of pear trees. They may impart precocity and dwarfing, influence fruit yield and quality, improve insect and disease resistance, and aid adaptation to wet or calcareous soils (Brooks, 1984; Westwood and Lombard, 1977). Evaluation of many pear genotypes as rootstocks with resistance to pear decline resulted in selection of clones *Pyrus betulifolia*, *P. calleryana*, and *P. communis* (Westwood et al., 1963). Several of these rootstocks were selected further by Melvin N. Westwood at Oregon State Univ., Corvallis, for their adaptability to Oregon conditions. 'Old Home' x 'Farmingdale 230' ('OH x F 230') (*P. communis*) is a semidwarf rootstock, 'Oregon Pear Rootstock (OPR) 157' (*P. calleryana*) is semivigorous, and 'OPR 260' (*P. betulifolia*) is vigorous. Although vigorous, *P. betulifolia* rootstocks produce high fruit yield, perform well in clay and poorly drained soils (Lombard and Westwood, 1987), and are resistant to pear decline and fire blight (Brooks, 1984). *Pyrus communis* rootstocks are winter hardy and

adaptable to the northern United States; *P. calleryana* and *P. betulifolia* are more suited to warmer climates such as California and southern Oregon. All three selections are graft-compatible with many pear cultivars and with *Cydonia oblonga* L. (quince). Propagation of these genotypes has not been easy and, therefore, has limited their availability.

Tissue culture methods are available for many pear species and cultivars (Berardi et al., 1993; Cheng, 1978; Dolcet-Sanjuan et al., 1990; Nicolodi and Pieber, 1989; Singha, 1980; Stimart and Harbage, 1989). Differences exist among the various genotypes for both basal medium and growth regulators. Our objectives were to evaluate Cheng and woody plant media (WPM) containing a range of BA, NAA, and IBA concentrations as potential media for shoot multiplication of 'OPR 157', 'OPR 260', and 'OH x F 230'. Following shoot multiplication, four in vitro and one ex vitro rooting treatment were tested. Shoots from rooting treatment were evaluated further for their ability to acclimate to greenhouse conditions.

## Materials and Methods

Stock cultures were grown on Cheng medium (Cheng, 1978). Growth-medium tests used Cheng medium and WPM (Lloyd and McCown, 1981). All media were adjusted to pH 5.2 with KOH/H<sub>2</sub>PO<sub>4</sub> before adding 3 g agar (Bitek, Difco, Detroit) and 1.25 g Gelrite (Schweitzerhall, South Plainfield, N.J.)/liter and autoclaving at 121C for 20 min. Standard growth room conditions were a 16-h photoperiod supplied by cool-white (Watt Miser, General Electric, Fairfield, Conn.) fluorescent bulbs (25  $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ ) at 25C.

Ecodormant branches were collected in February at the National Clonal Germplasm Repository (NCGR) at Corvallis, Ore., from mature 10- to 13-year-old, field-grown trees of 'OPR 157' (NCGR accession no. 1844), 'OPR 260' (NCGR no. 1379), and 'OH x F 230' (NCGR no. 1360) for forcing budbreak in the greenhouse. Shoots were pruned to 30 to 60 cm, washed in 40C water with detergent, rinsed under running tap water for 30 min, and placed in containers with 9 g Floralife (Floralife, Burr Ridge, Ill.)/liter in 30C water. Each week, 1 to 2 cm of the basal end of the branches were trimmed and the solutions were replaced. Leafy shoots were collected after 3 to 4 weeks in the greenhouse. A second set of explants was collected directly from field-grown trees in April.

Shoots (2 to 3 cm) were stripped of leaves, washed in soapy water and rinsed under running tap water for 5 min. Explants were disinfected in 10% commercial bleach (sodium hypochlorite 5.25%) with five drops of Tween 20/500 ml, shaken on a rotary shaker for 10 min, and rinsed 3 times in sterile deionized water. Single-node sections were transferred to 20 x 100-mm tubes with 10 ml of Cheng medium supplemented with 4.4  $\mu\text{M}$  BA (Sigma, St. Louis). Uncontaminated explants were transferred into Magenta GA7 (Magenta, Chicago) boxes with 40 ml of the same medium and subcultured at 3-week intervals.

Media tested were Cheng and WPM with factorial combinations of BA at 0, 2, 4, 8, and 13  $\mu\text{M}$  and NAA or IBA at 0, 0.5, 1.0, 2.0, and 4.0  $\mu\text{M}$ . The experiment was a three-factor (BA concentration, auxin concentration, and auxin type), randomized, complete-block design, with three blocks per treatment and five shoots per block (Magenta box) (total of 15 shoots). The total treatment period was 6 weeks with one transfer in the third week. Shoot proliferation was scored based on the number of usable shoots that were  $\geq 1$  cm long. Optimal shoot multiplication was based on plant appearance (greener leaves and minimal chlorosis), mean shoot height >1.2 cm, and proliferation rate. Factorial analysis and means separation were performed on data using MSTATC (Michigan State Univ.). Significance was recorded at  $P \leq 0.05$ .

Rooting treatments included either an auxin dip before planting in Cheng's medium without growth regulators or planting directly into a medium with 10  $\mu\text{M}$  NAA or IBA. Treatments included 1) a quick-dip treatment (15 sec) with 10 mM NAA or IBA dissolved in dimethyl sulfoxide. 2) Microcuttings were grown in medium with 10  $\mu\text{M}$  IBA in light or darkness for 1 week, then transferred to growth-regulator-free medium under standard growth room conditions. 3) Control microcuttings were placed directly into growth-regulator-free medium. The experiment was a two-factor (treatment x genotype), randomized, complete-block design consisting of three blocks per treatment with five shoots per block (Magenta box) repeated once (30 shoots total). The root length, callus size, number of roots per shoot, and percent rooting were measured. To test ex vitro rooting, the shoot base (2 cm height) was

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dipped into a 1 Dip'N Grow (1% IBA, 0.5% NAA, and 98.5% inert materials; Astoria-Pacific, Clackamas, Ore.): 1 water (v/v) solution, then planted directly into 200-ml pots filled with a peat-perlite mix. The treated shoots were placed under intermittent mist (32-sec mist per 64-sec interval) on an enclosed bench for 2 weeks, followed by another 2 weeks in the greenhouse (16C night/27C day). Treatment consisted of 15 shoots per genotype, and the experiment was repeated once (n = 30). The root length, callus size, number of roots per shoot, and percent rooting were measured after 4 weeks. All rooted and nonrooted shoots from the in vitro treatments were rinsed under tap water to remove adhering medium before transplanting to 800-ml pot bands filled with 1 peat : 1 perlite (v/v) mix. The shoots were placed in a mist bed for 2 weeks, then transferred to the greenhouse bench for another 2 weeks as for ex vitro rooting.

### Results and Discussion

More than 90% of the explants obtained from forced ecodormant branches were free of fungal or bacterial contamination, and >90% produced shoots. Explants derived from shoots collected in April from the field were discarded because of contamination or browning. *Pyrus calleryana* 'OPR 157' and *P. betulifolia* 'OPR 260' were difficult to establish in culture due to either heavy callusing of the basal end and leaf axils or browning of medium and explants, but these symptoms declined after six to seven subcultures.

Optimal multiplication in a micropropagation system is based on high multiplication rates, an overall healthy appearance, and enough height for easy transfer. Shoot elongation after establishment was good ( $\geq 1.2$  cm after 3 weeks) at all auxin concentrations at  $\leq 8$   $\mu\text{M}$  BA (data not shown).

*Pyrus betulifolia*. Shoot multiplication for *P. betulifolia* 'OPR 260' on Cheng medium was influenced by BA concentration and auxin type and concentration (Table 1). Interactions were not significant ( $P \leq 0.05$ ) (Table 2). Proliferation increased as BA levels increased with either auxin. At 13  $\mu\text{M}$  BA, many small shoots (<1 cm long) were induced, but these are not included in the means. Of the treatments, 0 to 1.0  $\mu\text{M}$  IBA or NAA produced the best shoot multiplication at all BA levels. Explants did not multiply on medium without BA. More new shoots were produced on explants cultured on medium with IBA than with NAA. Shoot multiplication was significantly lower with auxin concentrations of 2 and 4  $\mu\text{M}$  than at 0 and 0.5  $\mu\text{M}$ . With either medium the best shoot multiplication was obtained on 8 or 13  $\mu\text{M}$  BA with 0.5 or 1.0  $\mu\text{M}$  NAA (data not shown for WPM). Over all auxin levels, shoots grown on WPM produced more shoots than those on Cheng medium (mean 1.2 vs. 0.8), but without auxin, the media were equivalent. NAA at 2 and 4  $\mu\text{M}$  inhibited shoot multiplication compared to 0 and 0.5  $\mu\text{M}$ . Other studies with pear have used only BA and no auxin for multiplication of *P. betulifolia* (Dolcet-Sanjuan

et al., 1990; Nicolodi and Pieber, 1989). Based on shoot proliferation and overall appearance, including leaf size and color and shoot height, the best micropropagation medium for *P. betulifolia* 'OPR 260' was Cheng medium with 8  $\mu\text{M}$  BA and 0.5  $\mu\text{M}$  IBA.

'OPR 260' produced the most rooted shoots (42.8%), the highest number of roots per shoot, and the longest roots on medium with 10  $\mu\text{M}$  IBA (in darkness) (Table 3). Successful rooting with IBA at 10 or 32  $\mu\text{M}$  (Dolcet-Sanjuan et al., 1990) and 1 mM (200 mg-liter<sup>-1</sup>) (Nicolodi

Table 1. Means of shoot multiplication of *Pyrus calleryana* Oregon Pear Rootstock (OPR) 157, *P. betulifolia* OPR 260, and *P. communis* Old Home x Farmingdale 230 ('OH x F 230') on Cheng medium with BA and NAA or IBA.

Growth regulator ( $\mu\text{M}$ )	Auxin	Shoots per explant (no.)					
		Pear cultivar					
		OPR 260		OH x F 230		OPR 157	
2	0	0.4 ± 0.4*	0.9 ± 0.3	0.7 ± 0.5	0.6 ± 0.5	1.1 ± 0.5	0.2 ± 0.3
	0.5	0.5 ± 0.5	1.1 ± 0.4	1.1 ± 0.6	0.7 ± 0.5	1.8 ± 0.6	0.2 ± 0.3
	1.0	0.2 ± 0.3	0.5 ± 0.2	1.0 ± 0.6	0.5 ± 0.5	0.9 ± 0.5	0.2 ± 0.3
	2.0	0.1 ± 0.3	0.5 ± 0.3	1.0 ± 0.4	0.5 ± 0.5	1.3 ± 0.5	0.1 ± 0.3
	4.0	0.1 ± 0.2	0.8 ± 0.4	1.1 ± 0.6	0.5 ± 0.4	1.5 ± 0.5	0.0 ± 0.0
4	0	0.9 ± 0.5	1.2 ± 0.4	2.0 ± 0.6	1.2 ± 0.5	1.7 ± 0.6	1.1 ± 0.5
	0.5	0.7 ± 0.3	1.8 ± 0.5	2.4 ± 0.6	1.1 ± 0.5	2.1 ± 0.6	1.1 ± 0.5
	1.0	0.4 ± 0.4	1.5 ± 0.5	2.1 ± 0.7	1.0 ± 0.6	1.7 ± 0.5	1.0 ± 0.4
	2.0	0.2 ± 0	1.3 ± 0.6	2.4 ± 0.9	0.9 ± 0.6	1.5 ± 0.5	1.0 ± 0.5
	4.0	0.1 ± 0.2	1.3 ± 0.6	1.2 ± 0.6	0.7 ± 0.4	1.8 ± 0.6	1.4 ± 0.6
8	0	1.3 ± 0.6	1.7 ± 0.5	3.8 ± 0.8	3.0 ± 0.7	3.1 ± 0.6	2.5 ± 0.6
	0.5	2.0 ± 0.3	2.6 ± 0.5	5.7 ± 0.9	6.9 ± 0.8	3.0 ± 0.6	1.9 ± 0.6
	1.0	1.1 ± 0.4	2.4 ± 0.5	3.3 ± 0.7	4.1 ± 0.7	2.5 ± 0.6	1.6 ± 0.6
	2.0	0.9 ± 0.5	1.9 ± 0.6	3.3 ± 0.8	2.3 ± 0.8	1.8 ± 0.5	1.9 ± 0.6
	4.0	0.3 ± 0.4	2.1 ± 0.6	2.2 ± 0.7	1.5 ± 0.6	2.4 ± 0.6	1.5 ± 0.6
13	0	1.7 ± 0.5	2.0 ± 0.3	4.6 ± 0.6	4.1 ± 0.6	2.0 ± 0.6	1.5 ± 0.5
	0.5	1.9 ± 0.4	3.3 ± 0.6	6.6 ± 0.9	8.7 ± 0.8	2.3 ± 0.6	1.1 ± 0.5
	1.0	1.9 ± 0.6	2.7 ± 0.3	4.4 ± 0.5	5.3 ± 0.7	2.2 ± 0.6	1.2 ± 0.5
	2.0	1.2 ± 0.3	1.8 ± 0.5	4.1 ± 0.8	3.3 ± 0.8	1.3 ± 0.5	1.1 ± 0.6
	4.0	0.7 ± 0.3	2.4 ± 0.8	2.1 ± 0.7	2.3 ± 0.6	1.9 ± 0.6	1.0 ± 0.5

\*No multiplication at 0  $\mu\text{M}$  BA.

\*Means  $\pm$  se.

Table 2. Analysis of variance for means of shoot multiplication on Cheng medium.

Source	F value		
	OPR 260	OH x F 230	OPR 157
Auxin concentration (concn)	4.9**	73.6***	11.7***
BA concn	26.6***	293.4***	161.0***
Auxin x BA concn	0.5*	19.0***	3.8**
Auxin type	45.2***	8.8**	283.1***
Auxin concn x auxin type	1.4**	5.5**	6.9**
BA concn x auxin type	1.0**	8.6**	6.7**
Auxin x BA x auxin type	0.4**	2.6**	1.8**

\*\*\*, \*\*Nonsignificant or significant at  $P \leq 0.01$  or 0.001, respectively.

Table 3. Means of growth characteristics of *Pyrus calleryana* 'Oregon Pear Rootstock (OPR) 157', *P. betulifolia* 'OPR 260', and *P. communis* 'Old Home' x 'Farmingdale 230' ('OH x F 230') on Cheng medium with four rooting treatments.

Genotypes	Treatment			Shoots rooting or acclimatized (%)	Roots (no.)	Callus (cm)	Shoot length (cm)
	Auxin	Concn	Method <sup>1</sup>				
<i>P. betulifolia</i> OPR 260	NAA	10 mM	Dip	19.8 b <sup>1</sup>	0.2 b	0.2 a	0.2 a
	IBA	10 mM	Dip	17.5 b	0.3 b	0.3 a	0.2 b
	IBA	10 $\mu\text{M}$	Dark	42.8 a	1.0 a	0.2 a	1.2 a
	IBA	10 $\mu\text{M}$	Light	27.9 ab	0.5 ab	0.2 a	0.5 b
<i>P. communis</i> OH x F 230	NAA	10 mM	Dip	85.6 ab	8.4 a	0.6 b	2.0 a
	IBA	10 mM	Dip	81.1 b	6.9 ab	0.9 a	1.4 a
	IBA	10 $\mu\text{M}$	Dark	90.0 a	5.8 bc	0.5 b	1.4 a
	IBA	10 $\mu\text{M}$	Light	85.6 ab	4.2 c	0.6 b	2.1 a
<i>P. calleryana</i> OPR 157	NAA	10 mM	Dip		0.3 a	0.3 a	0.1 a
	IBA	10 mM	Dip		0.2 a	0.3 a	0.1 a
	IBA	10 $\mu\text{M}$	Dark		0.2 a	0.2 b	0.1 a
	IBA	10 $\mu\text{M}$	Light		0.1 a	0.3 a	0.2 a

<sup>1</sup>Dipped for 15 sec in auxin and grown with 16 h light for 3 weeks or grown for 1 week on medium with auxin either in darkness or 16 h light, then transferred to hormone-free medium in the light for 2 weeks.

\*Mean separation within columns within a genotype by Duncan's multiple range test at  $P \leq 0.05$ . Microcuttings not treated with auxin did not form roots or callus and did not acclimatize.

and Pieber, 1989) was reported for *P. betulifolia* cultivars.

*Pyrus communis*. The BA concentration  $\times$  auxin concentration  $\times$  type interaction was significant in the shoot multiplication of 'OH  $\times$  F 230', but multiplication was mainly influenced by BA  $\times$  auxin concentration, the BA  $\times$  auxin concentration interaction, and to a lesser extent auxin type (Table 2). The highest shoot multiplication was obtained with 8 and 13  $\mu$ M BA with 0.5  $\mu$ M auxin. Mean shoot proliferation was greater for plants grown on medium with NAA (2.8 shoots), rather than with IBA (2.5 shoots). The best multiplication was on 0.5  $\mu$ M IBA (8.7 shoots). Both NAA and IBA at 0.5  $\mu$ M improved shoot multiplication; higher concentrations were inhibitory. BA and NAA concentrations and medium type also influenced multiplication (data not shown for WPM). The BA  $\times$  NAA concentrations interaction was significant, with the best multiplication at 0.5  $\mu$ M NAA with 8 and 13  $\mu$ M BA. The BA level  $\times$  medium interaction was significant, with 8 and 13  $\mu$ M BA producing the most shoots on Cheng medium. NAA at >0.5  $\mu$ M inhibited shoot multiplication on both media. Cheng is a modified MS medium (Murashige and Skoog, 1962), and MS or modified MS with BA concentrations of 3.3 to 20  $\mu$ M produced successful multiplication of *P. communis* (Dolcet-Sanjuan et al., 1990; Lane, 1979). Our results indicated that 'OH  $\times$  F 230' shoots grown on 0.5  $\mu$ M NAA or IBA produced the best shoot proliferation and 2 and 4  $\mu$ M auxin inhibited multiplication. To our knowledge, there are no reports of IBA effects on *P. communis* multiplication. Some researchers tested NAA at concentrations <0.5  $\mu$ M and found no beneficial effects (Lane, 1979; Singha, 1980); others omitted auxin altogether (Dolcet-Sanjuan et al., 1990). Lane (1979) found that NAA (0.05  $\mu$ M) and gibberellic acid (1  $\mu$ M) were slightly inhibitory to shoot proliferation in 'Bartlett'. Based on shoot proliferation and overall appearance, the best micropropagation regime in this study for *P. communis* 'OH  $\times$  F 230' was on Cheng medium with 8  $\mu$ M BA and 0.5  $\mu$ M IBA. Although shoot count was highest at 13  $\mu$ M, the shoot height and appearance were best at 8  $\mu$ M.

'OH  $\times$  F 230' had the highest percentage (>80%) of rooting of the three genotypes, and it rooted well on all media (Table 3). Although 10  $\mu$ M IBA in darkness led to 90% rooted shoots, the number of roots per shoot were significantly fewer than with the 10-mm NAA dip. NAA stimulated rooting of *P. communis* cultivars at 8  $\mu$ M (Viseur, 1987) and 10  $\mu$ M (Lane, 1979; Singha, 1980). Other investigators have had success rooting *P. communis* cultivars with IBA at 1  $\mu$ M (Lane, 1979) and 10 or 32  $\mu$ M (Dolcet-Sanjuan et al., 1990). Lane (1979) found that 10  $\mu$ M NAA produced 70% more rooted shoots than 10  $\mu$ M IBA, which was considered toxic for 'Bartlett'. The largest callus for 'OH  $\times$  F 230' shoots was induced with a 10-mm IBA dip (0.9 cm), and it was larger than all other calli. More rooting occurred in 'OH  $\times$  F 230' shoots, which had the largest mean callus size in all treatments than in all the others. Calli induced by the 10-mm

IBA and NAA dips developed mainly around the point of root origin. More roots were produced per 'OH  $\times$  F 230' shoot with the 10-mm NAA and IBA dips than with other combinations, but root lengths were similar in all.

*Pyrus calleryana*. The greatest influences on shoot multiplication for *P. calleryana* 'OPR 157' were the BA concentration  $\times$  auxin type and auxin concentration  $\times$  auxin type (Table 2) interactions. Shoot multiplication was best at 8  $\mu$ M BA without IBA or with 0 or 0.5  $\mu$ M NAA. Overall, Cheng medium with 0 or 0.5  $\mu$ M NAA produced the most shoot proliferation; higher auxin levels were inhibitory. Shoot multiplication was highest at 8  $\mu$ M BA and declined at 13  $\mu$ M, mainly due to the production of many shoots <1 cm high. Optimum BA concentrations for various *P. calleryana* genotypes have varied from 0.5 to 20  $\mu$ M (Table 1) (Berardi et al., 1993; Dolcet-Sanjuan et al., 1990; Stimart and Harbage, 1989). Stimart and Harbage (1989) reported that 5  $\mu$ M IBA reduced the multiplicative effects of BA, but lower concentrations did not affect *P. calleryana* 'Bradford'. A similar inhibitory effect on multiplication occurred at concentrations >0.5  $\mu$ M NAA with 'OPR 157'. Berardi et al. (1993) reported that NAA at 0, 0.05, and 0.1 mg-liter<sup>-1</sup> (0, 0.025, and 0.05  $\mu$ M) did not affect shoot proliferation of *P. calleryana* seedlings. These concentrations were well below the optimal 0.5  $\mu$ M NAA for 'OPR 157'. Over all growth regulator combinations, shoots grown on Cheng medium multiplied significantly better than those on WPM, but at 0.5  $\mu$ M NAA and 8  $\mu$ M BA, the multiplication rates were equal (data not shown for WPM).

'OPR 157' shoots rooted poorly in all media with the highest percentage rooting (23.9%) obtained with the 10-mm NAA dip treatment (Table 3). The controls grown on basal medium did not root. Berardi et al. (1991) reported 2.5  $\mu$ M NAA (0.5 mg-liter<sup>-1</sup>) promoted rooting significantly better than 2.5  $\mu$ M IBA (0.5 mg-liter<sup>-1</sup>) for *P. calleryana* seedlings cultured on half-strength MS for 6 weeks. Dolcet-Sanjuan et al. (1990) induced high percentages of rooting of *P. calleryana* 'OPR 191' with a 15-sec dip in 10 mm IBA or by growing the shoots on medium with 10 or 32  $\mu$ M IBA for 7 days followed by transfer to medium with no growth regulators. Stimart and Harbage (1989) were unable to induce rooting of 'Bradford' with IBA (0, 4.92, 14.8, and 39.8 mM). Although we were able to induce rooting with NAA and IBA, the percentages were low. The number of roots per shoot and root length were low in all treatments. Comparison of these results with earlier data indicate a large amount of variation among genotypes of *P. calleryana*.

*Ex vitro rooting treatment*. Neither rooting nor survival occurred in any cultivar for the ex vitro rooting treatment (Dip 'N Grow) and subsequent acclimatization. We were interested in testing this technique because successful ex vitro rooting can save time and reduce costs, but to our knowledge, ex vitro rooting has not been reported for pear.

*Acclimatization*. All in vitro-rooted shoots survived after 4 weeks of acclimatization in

the greenhouse (Table 3). The best rooting treatments resulted in  $\leq$ 90% of micropropagated shoots rooting and subsequently developing into plants in the greenhouse. Shoots that did not root in vitro died during acclimatization. Rooting and survival were absent for the control shoots (not rooted) or those that did not root in vitro.

### Summary

Micropropagation systems for the three pear rootstocks were similar but not identical. Axillary buds of forced ecodormant branches of all genotypes initiated well on Cheng medium supplemented with 4.4  $\mu$ M BA. Cheng's medium with 8  $\mu$ M BA was the best for multiplication of all three genotypes, but auxin types varied. IBA and NAA concentrations >0.5 or 1  $\mu$ M inhibited the shoot multiplication in all three genotypes. For *P. betulifolia* 'OPR 260', the best shoot multiplication required 0.5  $\mu$ M IBA, and rooting was best on medium with 10  $\mu$ M IBA (in darkness) for 1 week. Multiplication for *P. communis* 'OH  $\times$  F 230' was best on medium with 0.5  $\mu$ M IBA and rooting with the 10-mm NAA or IBA dip treatments. Micropropagation for *P. calleryana* 'OPR 157' was good on Cheng or WPM with 8  $\mu$ M BA and 0.5  $\mu$ M NAA with the 10-mm NAA dip treatment for rooting. Acclimatization of rooted plantlets was successful with 2 weeks each in the mist bed and 2 weeks in the greenhouse. We believe this to be the first report of a comprehensive study of auxin in pear micropropagation media. Our results showing stimulatory effects of low concentrations and inhibitory effects of high concentrations indicate the importance of auxin concentration for pear micropropagation.

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