Screening *Pyrus* Germplasm for in Vitro Rooting Response

Barbara M. Reed¹

U.S. Department of Agriculture, Agricultural Research Service, National Clonal Germplasm Repository, 33447 Peoria Road, Corvallis, OR 97333-2521

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Abstract. Micropropagated shoots of 49 *Pyrus* species and cultivars and one selection of *Pyronia veitchii* (Trabut) Guillaumin were evaluated to test their responses to several in vitro rooting techniques. Auxin treatment was required for rooting in most cases. Eighteen of 50 accessions rooted $\geq 50\%$ with a 15-second, 10-mm IBA dip followed by growth on medium with no growth regulators (NGR). Twelve accessions rooted on a medium with 10 μM IBA applied for 1 week followed by NGR medium for 3 weeks; NGR medium alone was effective for only two accessions. Twenty-eight accessions rooted poorly with IBA treatments; an additional treatment of a 15-second dip in 10 mM NAA followed by NGR medium produced $\geq 50\%$ rooting for eight genotypes. Root production increased for 10 of 19 especially recalcitrant genotypes by 10 μM IAA treatments in darkness or at 30C and NAA dip treatments. Of rooted shoots, 73% survived acclimation in the greenhouse. Selections of *Pyrus betulifolia* Bunge, *P. calleryana* Decne., *P. hondoensis* Kikuchi and Nakai, *P. koehnei* C. Schneider, *P. pashia* Buch.-Ham. ex D. Don, *P. pyrifolia* (Burm.f.) Nakai cv. Shinseiki, *P. regelii* Rheder, *P. ussuriensis* Maxim., and the *Pyronia veitchii* selection failed to root in any of the treatments. Twenty-five of 32 *P. communis* L. cultivars and three other species rooted on at least one of the treatments. Chemical names used: 1-naphthaleneacetic acid (NAA), 1H-indole-3-butyric acid (IBA), 1H-indole-3-acetic acid (IAA).

Rooting pears (*Pyrus* spp.) in vitro has proven difficult, and scion cultivars are more difficult than those of rootstocks (Bhojwani et al., 1984). Several methods have been used to induce roots on micropropagated pears. Dolcet-Sanjuan et al. (1990) suggested placing shoots on medium with 10 μM IBA for 1 week or dipping them in 10 mM IBA for 12 sec, followed by placement on a hormone-free medium. On *P. pyrifolia*, NAA with 1,3,5-trihydroxybenzene (phloroglucinol) reduced basal callusing and increased rooting from 30% to 58% (Bhojwani et al., 1984). Rooting micropropagated shoots of *P. calleryana* cv. Bradford was unsuccessful with various IBA treatments (Stimart and Harbage, 1989), and *P. calleryana* cv. Oregon Pear Rootstock (OPR) 157 rooted poorly with IBA or NAA treatments (Yeo and Reed, 1995). *Pyrus calleryana* cv. OPR 191, however, rooted readily on standard rooting medium (Dolcet-Sanjuan et al., 1990). NAA produced good rooting of 'Seckel' pear with limited callus production on the cutting (Singha, 1980). *Pyrus communis* cultivars rooted successfully using either NAA or IBA (Rodriguez et al., 1991; Visser, 1987). Cheng (1979) used IBA at a range of concentrations for successful rooting of various fruit tree rootstocks. Collet (1988)

recommended the brief induction pretreatment of an IBA dip followed by transfer to hormone-free medium to induce the rooting stage for apple (*Malus domestica* Borkh.) and quince (*Cydonia oblonga* Mill.) cultivars. Although many approaches have been tried, there is no consensus as to the best method for rooting pear microcuttings, and great genotype differences have been observed. This study was designed to screen a large group of pear genotypes for response to techniques that produced roots on $>50\%$ of pear shoots in other studies.

Materials and Methods

In vitro plantlets of 50 accessions (49 *Pyrus* species and cultivars and one *Pyronia veitchii* selection), maintained on medium without growth regulators, were removed from cold storage (4C) and multiplied on Cheng's basal medium (Cheng, 1979) with (per liter) 1 mg N⁶-benzyladenine, 3 g agar (Bitek; Difco, Detroit, Mich.), and 1.75 g Gelrite (Schwizerhall, South Plainfield, N.J.) at pH 5.2. Growth regulators were obtained from Sigma Chemical Co., St. Louis. Cultures were grown in Magenta GA7 culture vessels (Magenta Corp., Chicago) at 25C under a 16-h photoperiod with 25 $\mu\text{m} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ irradiance provided by cool-white fluorescent bulbs.

Rooting treatments. Shoots were tested for rooting with the following treatments: 1) basal medium with no growth regulators (NGR) (4 weeks), which served as the control; 2) 15-sec dip in 10 mM IBA dissolved in dimethyl sulfoxide (Sigma) followed by growth on NGR medium (4 weeks); 3) 10- μM IBA medium (1 week) followed by NGR medium (3 weeks); 4) 15-sec dip in 10 mM NAA dissolved in DMSO followed by NGR medium (4 weeks);

5) treatment 3 in darkness (3 weeks) followed by light (1 week); 6) treatment 3 at 30C (4 weeks).

Screening. Initial screening was performed on all 50 accessions to determine the effect of treatments 1, 2, and 3. From each accession, nine shoots (20 to 25 mm long) were grown in each box with two replications per treatment ($n = 18$). Data were taken on percent rooting at 4 weeks. The number of roots produced, root length, and diameter of callus were determined for each shoot.

Twenty-eight accessions also were tested with treatment 4. Eighteen pears and the *Pyronia veitchii* accession were tested with treatments 5 and 6. Many of these accessions were chosen for further testing because they did not respond to the other treatments. Data were collected after 4 weeks. All treatments, tested on the groups of 28 (treatment 4) and 19 (treatments 5 and 6) accessions, were performed twice ($n = 18$). Analysis of variance and means comparison ($P \leq 0.05$) were performed using MSTAT software (Michigan State Univ., East Lansing).

Acclimatization. Rooted shoots of pear accessions with $\geq 50\%$ rooting frequency were transferred to the greenhouse for acclimatization and greenhouse culture. Rooted plantlets were washed in tap water to remove culture medium and were planted in perlite in a mist bed for 2 weeks, followed by 2 weeks in the greenhouse. Greenhouse conditions were $\approx 30\text{C}$ days/20C nights with 16-h days (natural lighting). Percent survival was noted 4 weeks after planting.

Results

Screen of 50 accessions. The 50 accessions tested showed wide variation in response to the first three rooting treatments (Table 1). Of 50 accessions, 28% rooted at $\geq 50\%$ on at least one of the three treatments; 22% failed to form roots. Twenty-two of the 50 accessions rooted ($\geq 50\%$) on one of the IBA treatments, and eight rooted on both. Averaged over all genotypes, growth on NGR produced roots on $<10\%$ of shoots; three times as many rooted with IBA in the medium (Table 2). The IBA dip treatment was significantly better ($P \leq 0.05$) than the control, but not significantly different from the 10- μM IBA treatment, indicating that the amount of IBA present was suitable for root induction in both IBA treatments. There were no significant differences in root length, roots per shoot or callus size between the IBA treatments. Callus formation, roots produced, and percent rooting tended to be positively associated in most of the pear genotypes.

Screen of 28 poor-rooting accessions. A group of 28 accessions, consisting mainly of poorly rooting ($<50\%$) cultivars, were tested for rooting response to a 15-sec, 10-mm NAA dip followed by NGR medium (Tables 1 and 3). For this group, NAA and IBA dips produced results better than NGR, but the results were not significantly different from each other. The IBA treatments produced similar results, but NAA was significantly better for promot-

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ing rooting than 10 µM IBA. Eight of the 28 accessions did not root with any treatment. Root length, roots per plant, and the amount of callus production were influenced by treatment. The best rooting treatments (NAA and IBA dip) generally produced more roots and more callus than less effective treatments.

Screen of 19 accessions with the entire treatment set. For 19 poorly rooting genotypes, the percentages of shoots rooting for the NGR and 10-µM IBA treatments were significantly lower ($P \leq 0.05$) than for the other treatments (Tables 1 and 4). Rooting on NGR was low (3.3%), and >16% of shoots rooted with the IBA dip, the NAA dip, the IBA in darkness, and the IBA at 30C. Only 27% of 19

accessions rooted $\geq 50\%$ with any of the treatments tested, and 22% did not root at all. Roots were longest with the IBA-dark and IBA-30C treatments, but roots per shoot were most abundant with NAA. The IBA-dark treatment produced significantly more callus than the others, and NGR produced the least. Rooting on 10 µM IBA in darkness was the best treatment for 'Admiral Gervais', 'Gliva Ukrainskaya/Wilenska Plenna', 'Madame du Ballet', and two accessions of *P. koehnei* (Table 1). 'Columbia', 'Fondante de Charneu', 'Kalle', and *P. elaeagnifolia* Pallas rooted well on 10 µM IBA at 30C.

Variation by genotype. The 39 *Pyrus* accessions that rooted were highly variable in

response to the rooting treatments (Table 1). Twenty-eight accessions, mostly *P. communis* cultivars, many originating in France, rooted best ($\geq 50\%$). Rooting percentages of 6% to 44% were found in 22 accessions; however, 11 accessions did not root with any of the treatments tested. Nine of the 11 nonrooting accessions were species other than *P. communis*, including *Pyrus betulifolia*, *P. calleryana*, *P. hondoensis*, *P. koehnei*, *P. pashia*, *P. pyrifolia* cv. Shinseiki, *P. regelii*, *P. ussuriensis*, and the *Pyronia veitchii* selection. Most of the pear accessions that rooted did so with 10 µM IBA, the IBA dip, or the NAA dip. Only occasionally did one of the other treatments prove more successful.

Acclimatization. Of in vitro-rooted shoots from 28 genotypes, 72% survived acclimatization and grew in the greenhouse (data not shown). Most genotypes had high acclimation survival (100% for 'Clapp Favorite', 'Colonel Wilder', 'Count A.W. Moltke', 'Fondante de Charneu', 'Bartlett', 'Kalle', 'Rosee de Juillet', 'Citron d'Ete', and 'Poirier Fleurissant Tard'), but a few, such as *P. cordata* Desv. and *P. ussuriensis*, died.

Discussion

In many genera, rooting of micropropagated shoots can be accomplished by removing cytokinins from the growth medium (Arrillaga et al., 1991; Kerns and Meyer, 1986). In pear and other woody plants, this approach is less successful than auxin treatments (Bhojwani et al., 1984; Dolcet-Sanjuan et al., 1990; Manzanera and Pardos, 1990). Poor rooting on hormone-free medium was confirmed by this screen, with only 9% of genotypes rooting without auxin treatment. IBA has been a successful rooting treatment for in vitro cultures (Collet, 1988; Corte-Olivares et al., 1990; Dolcet-Sanjuan et al., 1990; Manzanera and Pardos, 1990) and has produced $\geq 50\%$ rooted shoots in 44% of the pear genotypes that I tested. The production of roots on shoots treated with IBA was associated with increased callus production. The increased rooting that occurred with increased callus formation must be balanced with a rise in root fragility and increased susceptibility to fungal infection during acclimatization that may cause losses at transplanting (Banno et al., 1989).

Bhojwani et al. (1984) and Dolcet-Sanjuan et al. (1990) found that some genotypes responded better to NAA than to IBA. In this screen, individual genotypes, such as 'Admiral Gervais', 'Bergamote Tardive de Ganzel', 'Beurre d'Amanlis Panachee', and 'Kalle', responded better to NAA, but for others, NAA and IBA led to similar results (Table 1). In this study, following dark treatment, more callus formed at the base of pear shoots; this increase also was noted by Dolcet-Sanjuan et al. (1990).

Due to the variable rooting response in the genus *Pyrus*, it is difficult to predict which genotypes will root with a particular treatment. In many tree species in the Rosaceae (Nemeth, 1986), rooting has depended on genotype. Mature shoots of other woody species

Table 1. The effect of six rooting treatments on the percentage of shoots rooting in 39 *Pyrus* genotypes.

Accession	Shoots rooting (%) ^z					
	Treatment ^y					
	1	2	3	4	5	6
<i>P. communis</i> cultivars						
Admiral Gervais	0	11	0	33	33	0
Alliance Franco-Russe	0	22	28	11	---	---
Bartlett - Max Red	28	67	39	---	---	---
Bartlett - Striped	6	33	39	---	---	---
Belle Julie	6	50	17	---	---	---
Bergamote Tardive de Ganzel	33	28	6	78	---	---
Beurre d'Amanlis Panachee	6	22	33	78	---	---
Beurre Hardy	6	63	78	89	---	---
Butirra Precoce Morettini	0	0	44	---	---	---
Cascade	11	89	33	---	---	---
Citron d'Ete	22	56	22	---	---	---
Clapp Favorite	0	94	78	---	---	---
Colonel Wilder	17	67	94	---	---	---
Columbia	15	44	28	39	39	67
Count A.W. Moltke	6	89	50	---	---	---
Douglas	0	33	0	44	11	0
Fondante de Charneu	17	61	44	---	33	100
Gliva Ukrainskaya/ Wilenska Plenna	6	28	39	39	56	44
Kalle	22	44	50	89	33	72
Madame du Ballet	0	0	0	22	56	6
Marchland	17	44	6	56	---	---
MM-38 (rootstock)	0	33	11	22	---	---
Monchallard	6	72	50	33	---	---
Nijisseiki ^w x OH (decline susceptible)	61	72	89	---	---	---
Notaire Lepin	0	33	39	---	---	---
OPR-1 (rootstock)	22	11	56	---	---	---
Poirier Fleurissant Tard	39	78	39	---	---	---
Rosee de Juillet	6	61	22	---	---	---
Vineland 29018	0	33	50	---	---	---
<i>P. communis</i> rootstock (P-2366)(F.12-173)	0	61	11	56	0	0
<i>P. communis</i> rootstock (P-2462)(G.28-119)	0	39	61	100	---	---
<i>P. cordata</i> Desv.	0	72	6	---	---	---
<i>P. cossonii</i> Rheder	50	72	83	---	---	---
<i>P. dimorphophylla</i> Makino x <i>P. species</i>	0	61	11	78	---	---
<i>P. elaeagnifolia</i> Pallas (hybrid)	6	6	17	22	6	33
<i>P. koehnei</i> C. Schneider (808)	0	11	6	0	44	0
<i>P. koehnei</i> (812)	0	0	0	0	44	0
Good Christian ^v	39	89	72	---	---	---
Mien Suan Li ^u	0	11	0	0	11	0

^zBold type indicates highest rooting percentage.

^y1 = no growth regulator (NGR) medium (4 weeks), 2 = 10-mm IBA dip followed by NGR medium (4 weeks), 3 = 10-µM IBA medium (1 week) followed by NGR medium (3 weeks), 4 = 10-mm NAA dip followed by NGR medium (4 weeks), 5 = treatment 3 in darkness (3 weeks) then light (1 week), 6 = treatment 3 at 30C (4 weeks) (n = 18).

^uNot tested.

^w*Pyrus pyrifolia*.

^v*Pyrus pyrifolia* (Burm. f.) Nakai x *P. communis*.

^u*Pyrus ussuriensis* Maxim.

are also variable in their response to rooting treatments (Arrillaga et al., 1991). Micro-grafting of shoots onto seedling rootstocks may be the only option available for recalcitrant genotypes (Fridlund, 1989). Survival rates reported for transplanted, in vitro-grown pear shoots vary from 30% to 90% for *P. communis* cultivars (Marino, 1988; Rodriguez et al., 1991; Viseur, 1987) and 50% to 73% for *P. pyrifolia*

seedling-derived shoots (Bhojwani et al., 1984). Acclimation was highly successful for most of the pear genotypes screened.

Conclusions

The results of this screen mainly should serve as guides for more detailed comparisons of particular genotypes and for testing addi-

tional auxin concentration–duration combinations. Accordingly, I recommend that an IBA or NAA dip treatment be used for rooting pear genotypes of unknown rooting potential. The 10- μ M IBA (treatment 3) and 10-mm IBA dip (treatment 2) procedures were especially suitable for *P. communis* cultivars, with 10 mm NAA as an alternative for those that did not respond to IBA. For especially recalcitrant genotypes, treatment with 10 μ M IBA during 3 weeks in darkness or 4 weeks at 30C in the light are suggested.

Table 2. Effect of IBA treatments on mean percentage of shoots rooting, root length, roots per shoot, and callus diameter of 49 *Pyrus* and one *Pyronia veitchii* accession.

IBA treatment ¹	Mean ²			
	Shoots rooting (%)	Root length (mm)	Roots/shoot	Callus diam (mm)
1) None	8.9 b	4.5 b	0.2 b	0.2 b
2) 10 μ M, dip	35.3 a	6.9 ab	2.3 a	5.7 a
3) 10 μ M	27.0 ab	11.1 a	1.4 ab	2.8 ab
Source ³	Mean square	F value		
Percent rooting	9082.8	13.9***		
Root length	556.1	6.5**		
Roots/plant	53.2	15.6***		
Callus size	378.7	45.9***		

¹Mean separation within columns by Duncan's multiple range test at $P \leq 0.05$ ($n = 900$).

²1 = no growth regulator (NGR) medium (4 weeks), 2 = 10-mm IBA dip followed by NGR medium (4 weeks), and 3 = 10- μ M IBA medium (1 week) followed by NGR medium (3 weeks).

³Variation between treatments: two degrees of freedom.

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

Table 3. Effect of IBA and NAA treatments on mean percentage of shoots rooting, root length, roots per shoot, and callus diameter of 28 difficult-to-root pear accessions.

IBA treatment ¹	Mean ²			
	Shoots rooting (%)	Root length (mm)	Roots/shoot	Callus diam (mm)
1) None	4.3 c	2.1 b	0.1 c	0.3 c
2) 10 μ M IBA, dip	22.7 ab	4.1 a	1.2 b	4.7 ab
3) 10 μ M IBA	5.5 bc	5.8 a	0.8 bc	2.5 bc
4) 10 μ M NAA, dip	31.8 a	5.9 a	2.0 a	5.1 a
Source ³	Mean square	F value		
Percent rooting	3775.3	6.7***		
Root length	94.0	2.4 ^{ns}		
Roots/plant	18.5	8.1***		
Callus size	142.0	9.9***		

¹Mean separation within columns by Duncan's multiple range test at $P \leq 0.05$ ($n = 504$).

²1 = no growth regulator (NGR) medium (4 weeks), 2 = 10-mm IBA dip followed by NGR medium (4 weeks), 3 = 10- μ M IBA medium (1 week) followed by NGR medium (3 weeks), and 4 = 10-mm NAA dip followed by NGR medium (4 weeks).

³Variation between treatments: three degrees of freedom.

^{ns}, ***Nonsignificant or significant at $P \leq 0.001$, respectively.

Table 4. Effect of six rooting treatments on mean percent rooting, root length, roots per shoot and callus diameter of in vitro cultured shoots of 19 difficult-to-root pear genotypes.

IBA treatment ¹	Mean ²			
	Shoots rooting (%)	Root length (mm)	Roots/shoot	Callus diam (mm)
1) None	3.3 c	1.4 c	0.1 d	0.1 d
2) 10 μ M IBA, dip	16.4 a	3.1 b	1.0 b	2.8 b
3) 10 μ M IBA	10.3 b	3.4 b	0.5 c	1.2 cd
4) 10 μ M NAA, dip	20.3 a	3.2 b	1.4 a	3.2 b
5) 10 μ M IBA, dark	20.0 a	5.4 a	1.0 ab	4.9 a
6) 10 μ M IBA, 30 C	17.0 a	5.7 a	0.9 bc	2.3 bc
Source ³	Mean square	F value		
Percent rooting	806.2	1.7 ^{ns}		
Root length	49.0	1.2 ^{ns}		
Roots/plant	4.0	2.1 ^{ns}		
Callus size	52.2	7.6***		

¹Mean separation within columns by Duncan's multiple range test at $P \leq 0.05$ ($n = 342$).

²1 = no growth regulator (NGR) medium (4 weeks), 2 = 10-mm IBA dip followed by NGR medium (4 weeks), 3 = 10- μ M IBA medium (1 week) followed by NGR medium (3 weeks), 4 = 10-mm NAA dip followed by NGR medium (4 weeks), 5 = treatment 3 in darkness (3 weeks) then light (1 week), and 6 = treatment 3 at 30C (4 weeks).

³Variation between treatments: five degrees of freedom.

^{ns}, *** Nonsignificant or significant at $P \leq 0.001$, respectively.

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