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Micropropagation of 'Wild pear' *Pyrus pyrifolia* (Burm F.) Nakai. II. Induction of Rooting

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Abstract

The present study was undertaken to study the effects of cultural conditions, auxins and phloroglucinol on *in vitro* rhizogenesis in wild pear. Higher mean rooting percentage (28.78%) was obtained in solid medium, as compared to liquid medium (6.80%), irrespective of the growth regulator concentrations used. The rooting response was better with lower concentrations (0.125 and 0.25 mg l^{-1}) of NAA than IBA. At higher growth regulator concentrations (0.5-2.0 mg l^{-1}) though the rooting response was poor, yet significantly higher rooting was observed with IBA as compared to NAA. A combination of NAA and IBA resulted in significant improvement of rooting percentages over NAA or IBA alone. However, this improvement in rooting response was only at lower concentrations (0.125 and 0.25 mg l^{-1}) of auxin combination (NAA+IBA). The highest rooting (81.47%) was obtained on solid medium with NAA and IBA at 0.25 mg l^{-1} each followed by NAA and IBA 0.125 mg l^{-1} each (78.24%). 2,4-D failed to induce rooting. A higher number of roots (2.53) was obtained on solid medium, than on liquid medium (1.48). NAA resulted in higher number of roots per shoot than IBA, but at lower concentrations viz. 0.125-0.25 mg l^{-1}. The highest number of roots per microshoot (7.20) was obtained on liquid medium supplemented with NAA (0.25 mg l^{-1}). It was on a par with NAA + IBA (0.25 mg l^{-1} each) and IBA 0.25 mg l^{-1}. However, the latter treatments resulted in low rooting percentages and very poor root length. The root length in solid medium (1.18 cm) was significantly more than that on liquid medium. During the *in vitro* root induction some microshoots died of shoot-tip necrosis (STN). The STN percentages were significantly reduced on liquid medium in comparison to solid medium. Raising the Ca levels to 3 mM (T_1) significantly reduced the STN (2.60%). However, a further increase in Ca levels to 6 and 9 mM considerably increased the STN. The boron supplementations (200, 500 and1000 µM) reduced the STN to zero per cent and no symptoms of boron toxicity was observed even at 1000 µM. Rooting behaviour of the wild pear was significantly affected by the media phase and phloroglucinol. PG had a stimulatory effect on the rooting response of wild pear and the roots obtained on solid medium supplemented with PG were better than those on liquid medium. The highest *ex vitro* survival percentage of the wild pear plantlets (52.03 %) was observed on sand + soil (3:1 v/v) followed by 33.77 % on sand + soil (2:1 v/v) and 10.50% on perlite.

Keywords: in vitro rooting, culture conditions, wild pear

Introduction

In vitro adventitious root formation can be induced quite readily in many herbaceous species, but it can be very recalcitrant in most woody species. In case of micropropagation of woody species, stage III (induction of rooting) probably is the most difficult of the three stages to accomplish (Hu and Wang, 1983). To the best of our knowledge no other research workers has taken up the work on the micropropagation of 'wild pear' *Pyrus pyrifolia* (Burm F.) Nakai in India or abroad. Rooting *Pyrus* spp. *in vitro* has proven difficult (De Paoli, 1989 and Reed, 1995), and scion cultivars have proved more difficult to root than rootstocks (Bhojwani et al., 1984). However, several authors were successful in rooting European pears, but the results were poorer with Asian pears (Chevreau et al., 1992). While studying the *in vitro* rooting response of forty nine *Pyrus* species and cultivars, Reed (1995) failed to induce rooting in eight genotypes that included *P pyrifolia*, hence, reported it to be a difficult to root species. The type and concentration of auxin and cultural conditions influence the *in vitro* rooting. Addition of phloroglucinol (PG; 1,3,5, trihydroxy benzene), a phenolic compound, to culture media has stimulated *in vitro* rooting in some fruit crops belonging to family rosaceae including pear (James and Thurbon, 1979 and Berardi et al., 1991). The present study was undertaken to study the effects of cultural conditions, auxins and phloroglucinol on *in vitro* rhizogenesis in wild pear.

Materials and methods

The shoots obtained after shoot multiplication on MS medium (Murashige and Skoog, 1962) supplemented with 1.5 mg l^{-1} BA and 0.5 mg l^{-1} IBA were cultured on basal solid or liquid media supplemented with different concentrations of NAA or/ and IBA (Table 1) for *de novo* regeneration of adventitious roots. The *in vitro* raised shoots (2.5-

Table 1 Effect of phase of medium and growth regulator levels on *in vitro* rooting (%) and number of roots per microshoot in wild pear

Growth Regulator (mg l^{-1})	Rooting (%)			Number of roots per microshoot		
	Solid medium	Liquid medium	Mean	Solid medium	Liquid medium	Mean
NAA 0.125	70.98 (57.39)	30.97 (33.79)	50.97 (45.59)	4.89	6.10	5.49
0.25	70.14 (59.49)	35.03 (36.26)	54.59 (47.88)	5.01	7.20	6.10
0.5	33.34 (35.24)	0.00 (0.00)	16.67 (17.62)	4.26	0.00	2.13
1.0	5.10 (12.94)	0.00 (0.00)	2.55 (6.47)	3.41	0.00	1.70
2.0	0.18 (2.42)	0.00 (0.00)	0.09 (1.21)	1.60	0.00	0.80
IBA 0.125	59.10 (50.22)	0.00 (0.00)	41.48 (25.11)	3.91	0.00	4.65
0.25	63.66 (59.90)	23.87 (33.06)	46.73 (42.99)	4.25	6.50	5.37
0.5	47.00 (43.26)	0.00 (0.00)	23.50 (21.63)	3.13	0.00	1.56
1.0	9.07 (17.49)	0.00 (0.00)	4.54 (8.75)	1.43	0.00	0.71
2.0	2.13 (8.32)	0.00 (0.00)	0.09 (1.21)	1.09	0.00	0.54
NAA + IBA 0.125	78.24 (62.21)	0.00 (0.00)	39.12 (31.11)	5.09	0.00	2.54
0.25	81.47 (64.54)	37.99 (39.19)	60.72 (51.87)	5.52	7.10	6.31
0.5	22.10 (28.02)	0.00 (0.00)	11.05 (14.01)	3.40	0.00	1.70
1.0	4.30 (11.89)	0.00 (0.00)	2.15 (5.94)	1.01	0.00	0.50
2.0	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00	0.00	0.00
2,4-D 0.125	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00	0.00	0.00
0.25	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00	0.00	0.00
0.50	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00	0.00	0.00
Control	0.00 (0.00)	1.38 (6.72)	0.69 (3.36)	0.00	1.17	0.59
Mean	28.78 (27.02)	6.80 (7.84)		2.53	1.48	
LSD (0.05)	Media (A) : (0.67) Growth regulator (B : (1.44) A x B : (2.03)			Media (A) : (0.20) Growth regulator (B : (0.61) A x B : (0.86)		

Figures in parentheses are the transformed values

3.0 cm) were cultured on half strength MS solid or liquid medium supplemented with 3 per cent sucrose and 100 mg l^{-1} inositol in culture tubes containing 20 ml medium for the induction of rooting. In the solid medium 0.7 per cent agar was added. The effect of combined application of phloroglucinol (0, 40.80 and 160 mg l^{-1}) and NAA plus IBA (0.125-1.0 mg l^{-1} each) on *in vitro* rooting of microshoots was also studied. In all the rooting experiments the observations on percentage of rooted shoots, number of roots per shoot, length of the longest root and Shoot-tip necrosis percentage were taken after three weeks of culture. The rooted microshoots were transferred to plastic containers (400 ml) containing various potting mixtures after thorough washing. The containers were drenched

with Baiting (Carbendazim) @ 0.1 per cent and high humidity (90-100 %) was maintained around the plantlets.

To study the independent effects of B and Ca on shoot-tip necrosis (STN), supplementation of these ions at various concentrations in culture medium was evaluated. Half MS solid medium was modified with three levels of B (200, 500 and 1000 µM) and four levels of Ca (1.5, 3, 6 and 9 mM). Calcium chloride ($CaCl_2.2H_2O$) and Boric acid (H_3BO_3) were used as sources of Ca and B, respectively. Culture media were supplemented with 0.25 mg l^{-1} NAA and IBA. Stage II derived shoots of all the *Pyrus* genotypes were incubated on these media for four weeks. Observations were then made on the development of STN (%) in cultures.

All the experiments were carried out as completely randomized designs as described by Panse and Sukhatme (1954) and replicated at least thrice. Ten cultures at each concentration formed one replication. The significance of variation among the treatments was observed by applying 'F' test. The least significant difference (LSD) at p=0.05 was calculated by multiplying standard error with 't' value (p=0.05) at error degree of freedom to compare the means of the treatments. To study the combined effect of two factors, the data were subjected to two-way ANOVA analysis.

Results and discussion

In the present studies, significantly higher rooting percentage (28.78%) was obtained in solid medium as compared to liquid medium (6.80%), irrespective to the growth regulator concentrations used. The findings are contrary to the findings of Singha, (1982), Ochatt and Caso, (1984) and Lane, (1979a) who obtained higher *in vitro* rooting percentages in pear on liquid medium. The poor rooting response of wild pear on liquid medium may be ascribed to the higher degree of basal callusing (Table 2) and vitrification. Irrespective of the phase of media, the rooting response was better with lower concentrations (0.125 and 0.25 mg l^{-1}) of NAA than IBA. At higher growth regulator concentrations (0.5-2.0 mg l^{-1}) though the rooting response was poor, yet significantly higher rooting was observed with IBA as compared to NAA.

Better rooting response of wild pear with NAA is in conformity to the findings of Singha (1980) who preferred NAA over IBA for inducing roots in *P. communis*, cv. Seckel to avoid the basal callus formation. Bhojwani et al., (1984) and Reed (1995) also found that some pear genotypes rooted better on NAA than on IBA. Too high an auxin concentration in rooting media is undesirable as it will lead to reduction in rooting by inducing basal callus formation (Lane, 1979b) or by inhibiting the root elongation (Thimann, 1977). This may be the reason for poor rooting response of wild pear at higher auxin concentrations in the present studies (Table 2). A stronger inhibition of rooting by NAA may be responsible for the poorer rooting response of wild pear to higher NAA levels than to IBA as NAA is a stronger auxin than IBA.

A combination of NAA and IBA resulted in significant improvement of rooting percentages over NAA or IBA alone. However, this improvement in rooting response was only at lower concentrations (0.125 and 0.25 mg l^{-1}) of auxin combination (NAA+IBA). An improvement in rooting with NAA and IBA combination as compared to NAA or IBA alone may be due to complementary effect of NAA and IBA.

The interaction between phase of medium and growth regulator concentrations was also statistically significant. The highest rooting (81.47%) was obtained on solid medium with NAA and IBA at 0.25 mg l^{-1} each followed by NAA and IBA 0.125 mg l^{-1} each (78.24%). Irrespective of the phase of medium and concentration, 2,4-D failed to induce rooting. The high degree of callus induced by all the 2,4-D concentrations (Table 2) may be responsible for the inhibition of rooting in all the genotypes as 2,4-D was the strongest of all the three auxins tested for induction of *in vitro* rooting. Sriskandarajah and Mullins (1981) also reported inhibitory effects of 2,4-D on *in vitro* root induction in Granny Smith apple, even at low concentrations. Table 1 also shows that irrespective of the growth regulators and their concentrations, higher number of roots (2.53) was obtained on solid medium than on liquid medium (1.48). Failure of rooting in wild pear on most of the liquid media lead to a significant reduction in the mean number of roots on liquid medium. The failure of wild pear microshoots to root on liquid medium may be attributed to the higher degree of basal callusing (Table 1) and vitrification. NAA resulted in higher number of roots per shoot than IBA, but at lower concentrations viz. 0.125-0.25 mg l^{-1}. The higher number of roots on medium supplemented with NAA than with IBA has been earlier reported in *P. calleryana* (Berardi et al., 1993). There was a significant interaction between media phase (solid or liquid) and growth regulator concentration for number of roots per microshoot. The highest number of roots per microshoot (7.20) was obtained on liquid medium supplemented with NAA (0.25 mg l^{-1}). It was on a par with NAA + IBA (0.25 mg l^{-1} each) and IBA 0.25 mg l^{-1}. However, the latter treatments resulted in low rooting percentages (Table 1) and very poor root length (Table 2). This may be due to higher degree of basal callusing (Table-2) and vitrification as observed during the studies. In case of solid medium, fortified with NAA and IBA (0.25 mg l^{-1} each) produced the highest number of roots in wild pear. It was on a par with NAA + IBA (0.125 mg l^{-1} each) and NAA at 0.125 and 0.25 mg l^{-1} alone. The root mean length in solid medium (1.18 cm) was significantly more than that on liquid medium (Table 2). Higher degree of basal callus induction on liquid medium (Table 2) may be responsible for the poor root length in wild pear. In general the average root elongation decreased with increasing auxin concentration in the root induction medium (Table 2). At com-

Table 2 Effect of phase of medium and growth regulator levels on root length (cm) and incidence of shoot tip necrosis (STN) and on the degree of basal callus formation during in vitro root induction in wild pear

Growth regulator ($mg\ l^{-1}$)	Root length (cm)			Shoot tip necrosis (%)			Degree of basal callus formation	
	Solid medium	Liquid medium	Mean	Solid medium	Liquid medium	Mean	Solid medium	Liquid medium
NAA 0.125	2.51	0.52	1.51	6.09 (14.24)	3.00 (9.93)	4.54 (12.08)	-	++
0.25	2.46	0.41	1.43	6.77 (15.00)	3.30 (10.43)	5.03 (12.71)	-	++
0.5	1.49	0.00	0.74	7.20 (15.49)	4.10 (11.62)	5.65 (13.56)	++	+++
1.0	0.52	0.00	0.26	7.78 (16.15)	4.00 (11.51)	5.89 (13.83)	+++	+++
2.0	0.50	0.00	0.25	7.97 (16.39)	4.06 (11.60)	6.02 (13.97)	++++	+++
IBA 0.125	2.55	0.43	1.49	6.50 (14.71)	3.16 (10.21)	4.83 (12.46)	-	++
0.25	2.60	0.38	1.49	6.78 (15.02)	3.65 (10.98)	5.21 (13.00)	+	++
0.5	1.87	0.00	0.93	7.38 (15.72)	3.80 (11.16)	5.59 (13.44)	++	++
1.0	0.52	0.00	0.26	8.07 (16.46)	3.97 (11.40)	6.02 (13.93)	+++	+++
2.0	0.40	0.00	0.20	8.48 (16.89)	4.49 (12.21)	6.49 (14.55)	++++	+++
NAA + IBA 0.125	2.42	0.00	1.42	6.20 (14.33)	3.14 (10.12)	4.67 (12.23)	+	+
0.25	2.38	0.31	1.34	8.46 (16.87)	3.29 (10.32)	5.88 (13.60)	++	++
0.5	1.86	0.00	0.93	10.11 (18.50)	3.81 (11.22)	6.96 (14.86)	++	+++
1.0	0.32	0.00	0.16	14.20 (22.10)	30.96 (11.40)	9.08 (16.74)	+++	+++
2.0	0.00	0.00	0.00	22.17 (28.07)	6.35 (14.55)	14.26 (21.31)	++++	+++
2,4-D 0.125	0.00	0.00	0.00	15.60 (23.22)	10.89 (19.24)	13.24 (21.23)	+++	+++
0.25	0.00	0.00	0.00	19.17 (25.94)	12.92 (21.05)	16.04 (23.49)	++++	++++
0.50	0.00	0.00	0.00	22.37 (28.20)	14.13 (22.03)	18.25 (25.12)	++++	++++
Control	0.00	1.16	0.58	6.10 (14.28)	2.28 (8.63)	4.19 (11.46)	-	-
Mean	1.18	0.17		10.39 (18.29)	5.17 (12.61)			
LSD (0.05)	Media (A) : 0.10 Growth regulator (B : 0.31 A x B : 0.44			Media (A) : (0.52) Growth regulator (B : (1.61) A x B : (2.28)				

Figures in parentheses are the transformed values Degree of callus formation: + < 2mm; ++ 2 – 4 mm; +++ 4 – 8 mm; and ++++ > 8 mm

parative concentrations, NAA, IBA or NAA + IBA did not differ significantly for root length. During the *in vitro* root induction some microshoots died of shoot-tip necrosis (STN). Data presented in Table 2 reveal that during *in vitro* rooting the incidence of STN varied between 2.28-22.37 per cent. The STN percentages were significantly reduced on liquid medium in comparison to solid medium. Better uptake of nutrients by pear microshoots on liquid

Table 3 Effect of calcium and boron supplementation in ½ MS medium on the incidence of shoot-tip necrosis (%) in the *Pyrus* species

Treatment	Shoot-tip necrosis (%)
T_o Ca 1.5 mM + B 100 µM (Control)	9.12 (17.49)
T_1 Ca 3.0 mM + B 100 µM	2.60 (9.26)
T_2 Ca 6.0 mM + B 100 µM	56.10 (48.49)
T_3 Ca 9.0 mM + B 100 µM	65.33 (53.92)
T_4 Ca 1.5 mM + B 200 µM	0.00(0.00)
T_5 Ca 1.5 mM + B 500 µM	0.00 (0.00)
T_6 Ca 1.5 mM + B 1000 µM	0.00 (0.00)
LSD (0.05)	(2.09)

medium as compared to solid medium (Singha,1982) may be responsible for reduction of STN on liquid medium. Inhibitors present in agar have been shown to have adverse effects on plant growth (Kohlenbach and Wernicke, 1978). The auxin levels used to induce *in vitro* rooting also influenced the occurrence of STN significantly. In wild pear the incidence of necrotic apices increased with an increase in the concentration of auxins (IBA, NAA, NAA + IBA or 2,4-D). At similar concentrations there was no significant difference between IBA and NAA for the percentage of STN. Combination of NAA + IBA resulted in higher STN than IBA or NAA alone at 1.0 and 2.0 mg l^{-1} levels. 2,4-D resulted in highest incidence of STN. Literature on shoot tip necrosis is scanty and inconclusive. The few studies conducted on STN in *in vitro* cultures, concentrated on STN during *in vitro* shoot multiplication. In the present investigations, it was found that during shoot multiplication the STN can be effectively overcome by early subculture (data not presented). However, the death of microshoots due to STN during *in vitro* rooting was a very serious problem. Berardi *et al* (1991) studied the effect of auxins (IBA and NAA) on the incidence of STN during *in vitro* rooting of *P. calleryana*. They found that STN was not influenced by auxins.

Effect of calcium and boron supplementations on shoot-tip necrosis

Perusal of date in Table 3, show that Ca and B supplementations in the ½ MS medium, significantly affected the percentages of necrotic apices in wild pear. In wild pear, raising the Ca levels to 3 mM (T_1) significantly reduced the STN (2.60%). However, a further increase in Ca levels to 6 and 9 mM considerably increase the STN. A reduction in the incidence of STN in wild pear at 3 mM Ca may be ascribed to the higher uptake of Ca by the wild pear microshoots. Similar results have been reported in pistachionut (Abousalim and Mantell., 1994), quince (Singha et al., 1990) and potato (Sha et al., 1985). An increase in STN at higher Ca levels may be due to the toxicity of associated anion chloride. Raised levels of normal source of calcium in MS medium ($CaCl_2.2H_2O$) is reported to cause leaf yellowing, weak stem and death of tissues because of chloride toxicity (McCown and Sellemer, 1987). An increase in STN may also be due to higher Ca levels. Grigoriadou et al., (2000) have reported an increase in STN following an increase in Ca level (supplemented as Ca $(NO_3)_2$. $4H_2O$), in *P. communis*. The boron supplementations (T_4, T_5 and T_6) reduced the STN to zero per cent and no symptom of boron toxicity was observed even at 1000 µM. A high relative humidity is commonly observed in tissue culture vessels and this result in substantial reduction of transpiration and creates conditions in which calcium and boron related disorders might tend to develop in fast growing tips (Abousalim and Mantell, 1994). In this respect, an apparent reduction in STN of wild pear microshoots on relatively high boron concentrations (500 and 1000 µM) may be due to slow growth accompanied by possibly supraoptimal levels of boron which reduced STN as reported by Abousalim and Mantell (1994) in pistachionut.

Rooting behavior of wild pear was significantly affected by the media phase and phloroglucinol (Tables 4, 5 and 6). Therefore, the effect of phloroglucinol (PG) on the rooting response of pear genotypes was studied in solid as well as liquid media. Irrespective of the media phase, PG had a stimulatory effect on the rooting response of wild pear and the roots obtained on solid medium supplemented with PG were better than those on liquid medium.

In solid medium, the mean rooting percentage increased with an increase in PG level (Table 4). The highest rooting (61.68%) was observed at 160 mg l^{-1} and it was on a par with 80 mg l^{-1} PG in the rooting medium. Among the growth regulator combinations, the highest rooting (83.43%) was observed with NAA + IBA (0.25 mg l^{-1} each), irrespective of PG levels, followed by NAA + IBA (0.125 mg l^{-1} each) which resulted in 80.05 per cent rooting. However, at higher growth regulator levels the rooting response was poor. The PG and growth regulator levels interacted significantly. The highest rooting (86.29%) was observed with NAA + IBA (0.25 mg l^{-1} each), and PG (160 mg l^{-1}), which was on a par with NAA + IBA (0.25 mg l^{-1} each) and 80 mg l^{-1} PG level. In liquid medium, there was a considerable increase in rooting at all the three PG levels (Table 4). Irrespective of the growth regulator levels, highest rooting (88.80%) was attained with 40 mg l^{-1} PG which was on a par with 80 mg l^{-1} PG. However, there was a significant reduction in rooting percentage (83.53%) at 160 mg l^{-1} PG. The growth regulator levels also had a significant effect on rooting. The highest rooting (72.25%)

Table 4 Effect of supplementation of ½ MS solid medium and liquid media with varying levels of phloroglucinol (PG; mg l^{-1}) and growth regulators (mg l^{-1}) on per cent *in vitro* rooting

Phloroglucinol	Solid medium					Liquid medium				
NAA + IBA	PG 0	PG 40	PG 80	PG 160	Mean	PG 0	PG 40	PG 80	PG 160	Mean
0.125 each	78.24 (62.21)	79.26 (62.90)	81.01 (64.19)	81.69 (64.68)	80.05 (63.49)	0.00 (0.00)	90.43 (72.56)	85.00 (67.26)	80.13 (63.53)	63.89 (50.84)
0.25 each	81.47 (64.51)	82.27 (65.10)	83.68 (66.19)	86.29 (68.30)	83.43 (66.02)	37.99 (38.03)	90.10 (71.77)	87.9 (69.72)	85.0 (67.28)	75.25 (61.70)
0.5 each	22.10 (28.01)	43.61 (41.31)	46.32 (42.87)	48.12 (43.90)	40.04 (39.02)	0.00 (0.00)	89.00 (70.75)	88.90 (70.56)	87.00 (68.93)	66.22 (52.56)
1.0 each	4.30 (11.90)	25.12 (30.05)	27.20 (31.42)	30.61 (33.55)	21.81 (26.73)	0.00 (0.00)	85.70 (69.99)	81.10 (64.21)	82.00 (64.9)	62.2 (49.78)
Mean	46.53 (41.65)	57.26 (49.84)	59.55 (51.17)	61.68 (52.61)		9.50 99.50)	88.80 (71.27)	85.72 (67.94)	83.53 (66.19)	
LSD (0.05)	PG (A) : (1.53) Growth regulators (B) : (1.53) A x B : (3.07)					PG (A) : (3.35) Growth regulators (B) : (3.35) A x B : (6.70)				

Figures in parentheses are the transformed values

was observed with 0.25 mg l^{-1} NAA and IBA. Any deviations in this concentration of NAA and IBA resulted in significant reduction in rooting percentages. The growth regulator and PG levels interacted significantly. The highest rooting (90.43%) was recorded on the liquid medium supplemented with NAA and IBA (0.125 mg l^{-1} each) and 40 mg l^{-1} PG. It was on a par with all the other growth regulator levels at 40 mg l^{-1} PG and NAA + IBA (0.125 to 0.5 mg l^{-1} each) at 80 mg l^{-1} PG.

The number of roots per microshoot (Table 5) increased with an increase in PG level both in solid as well as liquid media. The highest number of roots (7.63) was observed with PG at 160 mg l^{-1} in the rooting medium. It was significantly higher than all other growth regulator levels. Irrespective of the PG levels, NAA + IBA at 0.25 mg l^{-1} each resulted in the highest number of roots per microshoot in wild pear. In liquid medium, all the three PG levels significantly increased the number of roots (Table 5) and root length (Table 6) over control. But, these three PG levels did not differ significantly for the number of roots per micro shoot and root length. Though on liquid medium, the rooting frequency of the microshoots was considerably improved, yet the roots were very small, tender and fragile. The PG levels had no significant effect on root length in solid media (Table 6).

Stimulation or inhibition of root initiation by phenolic compounds like PG is due to their interaction with auxins (Nemeth, 1986). The stimulatory effects of PG on *in vitro* rooting have been earlier reported in *P. callerya-na* (Berardi et al., 1991) and *P. pyrifolia* (Bhojwani et al., 1984). James and Thurbon (1979) also reported that in apple rootstock M9, addition of PG to the rooting medium improved rooting percentage. The anatomical study of roots and leaves has revealed that PG promoted xylem and chloroplast development (Jones et al., 1978). It has also been shown (James and Thurbon, 1981) that raised

Table 5 Effect of supplementation of ½ MS solid medium and liquid media with varying levels of phloroglucinol (PG; mg l^{-1}) and growth regulators (mg l-1) on number of roots per microshoot

Phloroglucinol	Solid medium					Liquid medium				
NAA + IBA	PG 0	PG 40	PG 80	PG 160	Mean	PG 0	PG 40	PG 80	PG 160	Mean
0.125 each	5.09	5.62	6.12	7.14	5.99	0.00	6.10	6.19	6.42	4.68
0.25 each	5.52	5.91	9.75	13.10	8.57	7.10	7.00	7.28	7.50	7.22
0.5 each	3.40	4.70	7.26	8.19	5.89	0.00	7.13	7.26	7.34	5.43
1.0 each	1.01	1.78	2.52	2.10	1.85	0.00	7.09	6.88	6.26	5.06
Mean	3.75	4.50	6.41	7.63		1.77	6.83	6.90	6.88	
LSD (0.05)	PG (A) : 0.97 Growth regulators (B) : 0.97 A x B : 1.93					PG (A) : 0.56 Growth regulators (B) : 0.56 A x B : 1.12				

Table 6 Effect of supplementation of ½ MS solid medium and liquid media with varying levels of phloroglucinol (PG; mg l^{-1}) and growth regulators (mg l^{-1}) on root length (cm)

Phloroglucinol	Solid medium					Liquid medium				
NAA + IBA	PG 0	PG 40	PG 80	PG 160	Mean	PG 0	PG 40	PG 80	PG 160	Mean
0.125 each	2.42	2.36	2.13	2.01	2.23	0.00	0.52	0.60	0.70	0.46
0.25 each	2.38	2.10	1.59	1.12	1.80	0.31	0.41	0.47	0.57	0.44
0.5 each	1.86	1.56	1.46	1.02	1.47	0.00	0.55	0.49	0.47	0.38
1.0 each	0.32	0.49	0.44	0.41	0.41	0.00	0.39	0.33	0.21	0.23
Mean	1.74	1.63	1.40	1.14		0.08	0.47	0.47	0.49	
LSD (0.05)		PG (A) : NS Growth regulators (B) : 0.50 A x B : NS					PG (A) : 0.12 Growth regulators (B) : 0.12 A x B : 0.24			

levels of endogenous PG in the microshoot favour the root initiation during the auxin sensitive phase and synergize with auxin, depending on the time of exposure (James and Thurbon, 1979). This action can be explained by the auxin-protector role of PG in a way that it may act as alternative substrate for IAA-oxidase and/or peroxidase, and this leads to increased levels of endogenous IAA (James, 1983). In plum and apple PG did not form a complex with auxin in the medium but was taken up directly and then 90 per cent PG is converted into phlorin, the β-glucoside of PG. Most of the label (supplied as ^{14}C-phloroglucinol) was found in the bases of the shoots (Marks et al., 1981). Zimmerman and Broome (1981) have reported that PG stimulated rooting in only one of the eight apple cultivars tried; with others it was either ineffective or inhibitory. The inhibition of rooting by PG in solid or liquid media may be due to the prolonged exposure of the microshoots to PG. The highest *ex vitro* survival percentage of the wild pear plantlets (52.03 %) was observed on sand + soil (3:1 v/v) followed by 33.77 % on sand + soil (2:1 v/v) and 10.50% on Perlite**.** The low *ex vitro* survival rates may be due to the lack of hardening chambers. The plants were hardened in normal polyhouses.

Conclusions

To the best of our knowledge, it is the first attempt to standardize the protocol for *in vitro* rooting of 'wild pear' [*Pyrus pyrifolia* (Burm F.) which is an important rootstock of pear in the subtropical pear industry of India. The wild pear microshoots rooted better on solid media as compared to liquid medium. NAA was more effective for root induction than IBA. The combination of NAA and IBA resulted in significant improvement in rooting frequency over NAA or IBA alone. Maximum rooting (81.47 %) was obtained on solid medium with NAA and IBA (0.25 mg l^{-1} each). During *in vitro* root induction some microshoots (2.28-22.37 %) died because of shoot-tip necrosis (STN). Raising the calcium levels to 3.0 mM in the nutrient medium significantly reduced STN in the microshoots. Supplementation of nutrient medium with 200, 500 and 1000 µM boron reduced the incidence of STN to zero per cent. Supplementation of rooting medium (solid or liquid) with phloroglucinol (PG) had a stimulatory effect on the rooting response, but on liquid medium the plants were vitrified with very small roots. However, on solid medium fortified with NAA + IBA (0.25 mg l^{-1} each) and PG (160 mg l^{-1}), 86.29 per cent rooting was obtained. The highest *ex vitro* survival percentage of the wild pear plantlets (52.03 %) was observed on sand + soil (3:1 v/v).

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