Indian Journal of Agricultural Sciences **82** (3): 225–9, March 2012

In vitro propagation of *Pyrus communis* cv. Bartlett

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Received: 9 March 2011; Revised accepted: 24 November 2011

ABSTRACT

Shoot tip explants derived from forced and unforced explants were subjected to different sterilization regimes. Satisfactory culture asepsis (61.60%) and survival (45.00%) was obtained with 0.1% mercuric chloride when forced explants were used. MS medium supplemented with BAP+IBA (1.5+0.01mg/l) resulted in maximum explant establishment (46.66%). Maximum proliferating cultures (52.66%) and shoot number/explant (8.73) was recorded in MS medium supplemented with BAP+IBA (2.00+0.01mg/l). Microcuttings (10-15mm) from the proliferated cultures were inoculated in two media supplemented with different IBA concentrations for rooting. MS medium fortified with IBA (1.0 mg/l) resulted in maximum rooting (46.66%), root number/shoot (4.26) and root length (36.50 mm).

Key words: Bartlett, Forcing, *In vitro* propagation, *Pyrus communis*

Pear (*Pyrus communis* L.) is grown in all the temperate zones of the world. It is the main species of commerce in Europe, North America, South America, Africa and Australia. In India, major pear-growing areas are located in Jammu and Kashmir, Himachal Pradesh, Punjab, Uttaranchal, Arunachal Pradesh, Manipur, Mizoram, Nagaland and Tamil Nadu. In Jammu and Kashmir pear crop occupies an area of 11,200 ha with the production of 43,080 million tonnes and productivity of 3846 million tonnes/ha (Department of Horticulture 2007).

Budding and grafting are conventional methods of pear propagation, but these methods are slow, cumbersome and season dependant. Micropropagation is the only rapid asexual multiplication method available for mass multiplication of commercial cultivars. In pear, micropropagation was achieved for the first time in 1979. Since these first reports, significant progress has been made in the different areas of *in vitro* culture of pear (Anirudh *et al*. 2008, Bell *et al*. 2009). In view of the importance of pear in the national economy of India in general and Jammu and Kashmir in particular, large-scale multiplication of desired and commercial cultivars is the need of hour. To meet the growing demand of quality planting material the present investigation was laid to develop a commercial micropropagation protocol of *Pyrus communis* cv. Bartlett.

MATERIALS AND METHODS

Present studies were carried out in the Biotechnology Laboratory, Division of Pomology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir during 2008–10. Hardwood cuttings were collected from mature field grown stock plants of pear cv. Bartlett in November–December 08. Cuttings were treated with 0.2% Captan and stored in a cold store (refrigerator) in polyethylene bags until use. Cuttings were taken out of the cold storage after 45 days and placed in glass jars filled with distilled water after recutting their base. Cuttings were kept in incubation chamber maintained at 24 ± 1^0C. Sprouting of buds took place within 15–20 days. Shoots put forth by these sprouted buds served as forced explant source. Unforced explants were obtained from field grown mature trees of pear growing at experimental farm. Shoot tips colleted from forced and unforced explant sources were surfaced sterilized with different sterilants, alone or in combinations. Surface sterilized explants were rinsed 4–5 times with double distilled sterile water, prepared and cultured in MS basal medium. Data relating to culture asepsis and survival was noted after two weeks of inoculation.

Healthy explants were transferred onto fresh media for establishment studies using two different media, viz Murashige & Skoog's and Woody Plant medium supplemented with different growth regulators. Data relating

Based on complete information of MSc of the first author submitted to SKUAST (K) during 2010.

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to establishment and explant necrosis were recorded after 4±1 week of culture inoculation.

Well established cultures were transferred to fresh proliferation media fortified with different levels of growth regulators. Data pertaining to per cent proliferated cultures, number and length of shoots/explant were recorded after 4±1 weeks of inoculation in the proliferation media. Microshoots (10–15 mm) from proliferating cultures were aseptically excised and transferred to different media fortified with various concentrations of IBA for rooting. Rooting per cent, root number and root length were recorded after 4±1 weeks of inoculation in the rooting media. Experiments were laid out in completely randomized design with three replications and ten explants/treatment/replication. Data was statistically analyzed using Mini-Tab Statistical Package. To satisfy model assumptions of experiments, percentage data was subjected to angular transformations as suggested by Steel and Torie (1981).

RESULTS AND DISCUSSION

Forced and unforced shoot tip explants were subjected to five different sterilization regimes using MS as basal medium. Main effect of sterilization regimes on culture asepsis and survival was significant (Table 1.) Maximum aseptic cultures (59.68%) were recorded under S_5 (0.1% mercuric chloride for 10 min. + 70% ethyl alcohol for 10 sec.), followed by S_2 (0.1% mercuric chloride for 10 min.), whereas lowest aseptic cultures (35.35%) were recorded with S_3 (70% ethyl alcohol for 10 sec.). However maximum culture survival (46.38%) was recorded with S_2. Explant source had a significant effect on culture asepsis and survival (Table 1). Maximum culture asepsis (50.02%) and culture survival (29.46%) was recorded when explants were collected from cuttings forced under culture room (24±1^{0}C, 16/8 photoperiod and 3000 Lux). Combinations of sterilants increased the culture asepsis but with a decrease in culture survival. These results are in close conformity with studies of Dalal *et al*. (2006) who reported maximum culture asepsis and lower survival with combined treatments of sterilants. Higher culture asepsis and survival under forced explants may be due to relatively lesser disease inoculum load compared to field grown stock plants which are exposed to attack by different microorganisms. Amin (2005) and Rather (2010) have also reported similar results with forced explants of different crops.

Culture establishment media is useful for conditioning and adapting the explant to heteromorphic mode of nutrition and for stimulating its initial growth. Media and growth regulators had a significant effect upon the establishment of forced explants of pear (Table 2). Explant establishment increased with increase in BAP concentration and was recorded maximum (43.50%) with BAP + IBA (1.5 + 0.01 mg/l). Further increase in BAP concentration had a negative effect on explant establishment. MS medium was found

Table 1 Influence of various sterilants on culture asepsis (per cent aseptic culture) and survival (%) in forced and unforced explants of pear cv. Bartlett

Sterilant	Culture asepsis (%) Explant source			Explant survival (%) Explant source		
	Unforced	Forced	Mean	Unforced	Forced	Mean
S_1	24.66 (34.21)	33.33 (39.38)	29.00 (36.79)	19.00 (25.56)	29.00 (32.21)	24.00 (28.88)
S_2	56.66 (53.92)	66.66 (59.68)	61.66 (56.80)	49.00 (43.00)	59.00 (49.77)	45.00 (46.38)
S_3	23.33 (31.32)	33.33 (39.28)	28.33 (35.35)	15.66 (22.85)	22.33 (27.77)	18.99 (25.31)
S_4	43.30 (45.90)	50.00 (50.00)	46.66 (40.02)	12.33 (20.14)	12.33 (20.14)	12.33 (20.14)
S_5	63.33 (57.71)	70.00 (61.60)	66.66 (59.68)	12.33 (20.14)	9.00 (17.44)	10.66 (18.79)
Mean	42.72 (44.68)	50.66 (50.02)		21.66 (26.33)	76.33 (29.46)	
CD (*P*=0.05)						
Main effect of explant origin			3.09	2.44		
Main effect of sterilants			4.29	3.86		
Explant origin × sterilants			6.07	5.46		

Figures in parentheses are angular transformed values

S_1, 10% sodium hypochlorite for 10 min.; S_2, $HgCl_2$ (0.1%) for 10 min.; S_3, Ethyl alcohol for 10 sec.; S_4, S_1+S_3; S_5, S_2+ S_3

superior to WPM recording maximum explant establishment of (32.53%). Interactions between growth regulators and media were found significant recording maximum establishment of 46.68 per cent when MS medium was supplemented with BAP + IBA (1.5+ 0.01 mg/l). Significant effect of growth regulators and media was also observed on explant necrosis (Table 1). Maximum necrotic cultures (19.08%) were recorded when explants were cultured in WPM and minimum (13.00%) when cultured in MS medium. Higher concentration of growth regulators resulted in decrease in explant necrosis. Minimum necrotic cultures (7.50%) were obtained with BAP + IBA (2.00 + 0.01 mg/l). There was significant interaction between media and growth regulators in reducing the explant necrosis which was recorded minimum (5.00%) when MS medium was supplemented with BAP + IBA (2.00 + 0.01 mg/l). These results are in close conformity with results of Predieri, *et al*. (1989) who reported that there was problem of apical necrosis in pear cv. Conference and percentage increased when BAP was used at lower concentration. These results are contrary to findings of Changzhu *et al*. (2002) who reported that shoot tip necrosis in pear was related to duration of *in vitro* cultivation and BAP has no effect on shoot tip necrosis.

Established cultures were transferred to fresh media fortified with different concentrations of BAP and IBA for shoot proliferation through stimulation of axillary buds. Effect of various growth regulators and media on proliferation was significant (Table 3). Percentage of proliferating cultures and number of shoots/explant increased with increase in BAP concentration. Maximum proliferating cultures (47.33%) and highest shoot number/explant (8.38) was obtained with BAP + IBA (2.00 + 0.01 mg/l). However, this treatment recorded minimum shoot length (13.70 mm). Cytokinins are known to promote cyto-differentiation (Fukuda and Komamine (1985) and eliminate apical dominance (Wickson and Thiamann 1958) thereby promoting axillary shoot proliferation. BAP has been found to be most effective cytokinin for stimulating axillary shoot proliferation, followed by kinetin and 2-iso pentenyladenine (Hu and Wang 1983). Results in the present studies are in close conformity with Anirudh and Kanwar (2008) who reported a positive correlation between BAP and shoot multiplication up to a certain level in pear. Decrease in shoot length with increase in BAP concentration is in close proximity with Stimart and Harbage (1989).

MS media proved superior to WPM recording maximum proliferated cultures (34.25%), shoot number/explant (5.61) and shoot length (22.56 mm). Anirudh and Kanwar (2008) also reported higher rate of multiplication on MS medium supplemented with BAP in pear. Combination of cytokinin and auxin for better proliferation in the present studies are in close conformity with Sedlak *et al*. (2003) who obtained higher shoot proliferation and multiplication in different *Pyrus* species with combination of cytokinin BAP and auxin (IBA).

Microshoots (10–15 mm) from proliferating cultures were aseptically excised and transferred to different media fortified with various concentrations of IBA for rooting. Amin (2005) has also reported maximum rooting with shoots equivalent to or exceeding 10 mm in almond. Media and growth regulators had significant effect on all rooting parameters (Table 4). Maximum percentage of rooted shoots (41.68%) with maximum root number (4.24) was recorded when IBA was used at a concentration of 1.00 mg/l. Higher concentration decreased both these parameters. However, root length increased with increase in IBA concentration and was recorded maximum (28.10 mm) with IBA (2.00 mg/l). All rooting parameters were better when MS medium was

Table 2 Influence of growth regulators and media on explant establishment and necrosis in pear (*Pyrus communis*) cv. Bartlett

Growth regulator	Explant establishment (%)			Necrosis (%)		
BAP+IBA (mg/l)	M_1	M_2	Mean	M_1	M_2	Mean
0.50+0.01	22.00	18.33	20.16	18.00	23.33	20.66
	(27.96)	(25.34)	(26.65)	(25.10)	(28.87)	(26.99)
1.0+0.01	29.33	25.33	27.33	16.00	27.66	21.83
	(32.79)	(30.21)	(31.50)	(23.57)	(31.10)	(27.33)
1.50+0.01	46.66	40.33	43.50	13.00	15.33	14.16
	(43.08)	(39.42)	(41.25)	(21.12)	(23.05)	(22.08)
2.0+0.01	33.33	30.33	31.03	5.00	10.00	7.50
	(35.26)	(33.41)	(34.34)	(12.92)	(18.43)	(15.67)
Mean	32.53	28.56		13.00	19.08	
	(34.77)	(32.07)		(20.40)	(25.36)	
CD (*P*=0.05)						
Growth regulators(GR)			0.64			4.27
Media (M)			0.91			2.70
GR × M			1.29			6.04

Figures in parentheses are angular transformed values

Table 3 Influence of growth regulators and media on proliferation in pear (*Pyrus communis*) cv. Bartlett

Growth regulator	Proliferating cultures (%)			Number of shoots/explant			Shoot length (mm)		
BAP+IBA (mg/l)	M_1	M_2	Mean	M_1	M_2	Mean	M_1	M_2	Mean
0.50+0.01	14.00 (21.94)	12.00 (20.26)	13.00 (31.23)	2.45	2.03	2.24	24.15	20.55	22.35
1.0+0.01	29.66 (32.99)	24.33 (29.55)	27.00 (31.27)	4.36	3.80	4.08	26.95	25.85	26.40
1.50+0.01	40.66 (39.62)	37.00 (37.45)	38.83 (38.53)	6.90	6.20	6.55	24.30	20.70	22.50
2.0+0.01	52.66 (46.53)	41.66 (40.19)	47.33 (43.46)	8.73	8.03	8.38	14.85	12.55	13.70
Mean	34.25 (35.27)	28.75 (31.86)		5.61	5.01		22.56	19.91	
CD (P=0.05)									
Growth regulators(GR)			2.35			0.09			0.16
Media (M)			1.66			0.06			0.11
GR × M			3.33			0.13			NS

Figures in parentheses are angular transformed values

Table 4 Influence of growth regulators and media on rooting in pear (*Pyrus communis*) cv. Bartlett

IBA	Rooting (%)			Number of roots/shoot			Root length (mm)		
(mg/l)	M_1	M_2	Mean	M_1	M_2	Mean	M_1	M_2	Mean
0.50	33.33 (35.21)	26.66 (30.99)	30.00 (33.10)	3.20	3.13	3.16	17.00	12.50	14.70
1.00	46.66 (43.07)	36.66 (37.22)	41.66 (40.15)	4.26	4.23	4.24	36.50	32.50	24.50
1.50	16.66 |(23.88)	13.33 (21.14)	15.00 (22.50)	1.66	1.60	1.63	28.00	23.50	25.70
2.00	10.00 (18.43)	10.00 (18.43)	10.00 (18.43)	1.26	1.23	1.24	29.40	26.50	28.10
Mean	26.66 (30.14)	21.66 (26.95)		2.59	2.54		27.80	23.70	
CD (P=0.05)									
IBA			6.11			0.08			0.16
Media (M)			4.32			0.02			0.11
IBA × M			8.60			0.10			NS

*Figures in parenthesis are angular transformed values

used. The results obtained are in close conformity with Pasqual *et al*. (2002) in *Pyrus betulaefolia.* and Stimart and Harbage (1989) in *Pyrus calleryana*.

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