MICROPROPAGATION OF PEAR (PYRUS SPP.): A REVIEW

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ABSTRACT

In pear, micropropagation was achieved for the first time in 1979 on pear rootstock ‘OH x F 51’ and scion variety ‘Bartlett’. Significant progress has been made in the different areas of in vitro culture of pear since these first reports. In most cases, Murashige and Skoog (MS) revised medium (1962) was used as mineral medium for culturing many Pyrus species and cultivars for regeneration and/or proliferation, subculturing and subsequent rooting. The lower concentration of major elements in WPM (Woody Plant Medium; Lloyd and McCown, 1980) was more suitable for micropropagation of some pear genotypes. Starting material for establishment of pear in vitro culture consists mostly of shoot tips or single node explants from grafted plants. BAP is the most frequently used cytokinin for pear micropropagation. Exogenous auxins do not promote axillary shoot proliferation, however, culture growth may improve by their presence. Rooting Pyrus spp. in vitro has been proven difficult, and scion cultivars have proved more difficult to root than rootstocks. However, several authors were successful in rooting European pears, but the results were poorer with Asian pears. NAA is mostly used for inducing rooting followed by IBA, IAA and 2, 4-D. Brief exposure of shoots to auxin and darkness followed by their transfer to hormone free medium resulted in good rooting response in pear. The success of transplanting and survival of plants greatly depends on the quality of roots. In pear, acclimatization has been accomplished in various substrates by progressively decreasing the relative humidity.

Pears are grown in all the temperate zones of the world. In 2007, the world’s annual production of pear was 1,95,34,480 mt with a total area of 20,32,089 ha under cultivation. In India, pear is grown from warm humid subtropical plains to cold dry temperate regions and occupying an area of 23,000 ha with an annual production of 2, 00, 000 mt (Anonymous, 2006). The chief pear growing areas are located in Jammu and Kashmir, Himachal Pradesh, Punjab, Uttaranchal, Arunachal Pradesh, Manipur, Mizoram, Nagaland and Tamil Nadu. The genus Pyrus belongs to subfamily Pomoideae in the family Rosaceae and contains twenty two species. The genus is supposed to have originated in the montaneous region of Western China from a double hybrid between two ancestral species with basic chromosome numbers of X=8 and X=9. All Pyrus species are diploid (2n=34, X=17). A few polyploid cultivars of P. communis L. also exist.

The domestic pears of Asia are derived mostly from P. pyrifolia (Burm F.) Nakai, known as Japanese or sand pear. P. communis L. is the main species of commerce in Europe, North America, South America, Africa and Australia. Most of the soft pear varieties being cultivated in the temperate region of India also belong to P. communis L. The sand pear, P. pyrifolia is the main cultivated species in southern and central China, Japan. Sand pear is also widely cultivated in India.

Tissue culture techniques have been used for rapid plant propagation, plant breeding and for studying various aspects of plant growth and development in pear. Micropropagation is the rapid asexual in vitro multiplication of a desired plant. In pear, micropropagation was achieved for the first time in 1979 on pear rootstock ‘OH x F 51’ (Cheng, 1979) and scion variety Bartlett (Lane, 1979a). Since these first reports, significant progress has been made in the different areas of in vitro culture of pear. The literature relevant to micropropagation and somatic cell culture in Pyrus spp. is being reviewed as under.
**Nutrient medium**

In most cases, Murashige and Skoog (MS) revised medium (1962) was used as mineral medium for culturing many Pyrus species and cultivars for regeneration and/or proliferation, subculturing and subsequent rooting (Bell and Reed, 2002). A number of carbohydrates have been used in fruit tissue culture, but the sucrose has been most popular and versatile. Carbohydrates affect the growth and the frequencies of shoot developed and shoot proliferation of woody Rosaceous species. Sorbitol produced a good result for shoot multiplication of *P. pyrifolia* as compared to sucrose and fructose (Kadota et al., 2001). Sorbitol at 60 mM was the most effective carbon source for shoot proliferation in *P. pyrifolia* cv. Hosui (Kadota and Niimi, 2004). In *P. calleryana* L., by increasing sucrose concentration to 38 g l⁻¹ increased shoot number and by increasing sucrose to 60 g l⁻¹ increased shoot length and quality (Pasqual et al., 2002a).

In *P. communis*, cv. Seckel increasing agar concentrations resulted in decreased shoot growth, but shoot proliferation rate was significantly higher at 0.6 % agar as compared to 0.3 % or lower agar concentrations (Singha, 1982). Kadota et al. (2001) recommended that low concentrations of agar (0.4 and 0.6%) must be avoided for proliferating *P. pyrifolia* as it increased vitrification at low concentrations. However, 0.8 per cent agar had a better effect than 1.0 and 1.2 per cent agar for shoot proliferation. Leite et al. (1997) studied the effect of gelling agents on proliferation and growth of pear shoots. The best shoot proliferation and growth were obtained with explants cultured on medium containing Cialgas agar at any one of the concentrations (0.6-1.2%) and with Difco agar at 0.6 or 0.8 per cent. Gelrite induced vitrification. Zimmerman et al. (1995) advocated the use of a mixture of maize starch and Gelrite as a gelling agent for micropropagation of *P. communis*, cv. Anjou and Seckel supplemented with a polysaccharide hydric control agent to eliminate hyperhydricity. Damiano et al. (2000) increased the multiplication rate of wild pear (*P. communis* var. *pyraster* L.) by temporary immersion technique involved complete immersion of the proliferating explants in a liquid medium (containing benzyladenine and IBA), alternating with a dry period. Lucyszyn et al. (2006) have found improved in vitro shoot formation and rooting in pear (*Pyrus communis* L. cv. "Durondeau") with partial substitution of agar by galactomannans (GMs) extracted from seeds of *Cassia fistula* (cassia) or *Cyamopsis tetragonolobus* (guar gum, a commercial GM) at a significantly lower cost.

Many researchers have completely eliminated the use of gelling agent for their cultures by using shake or roller drum cultures and/or by using the so-called Heller bridges made of filter papers and supported in liquid medium. Lane (1979b) reported that cultures of *Prunus cistena* did not root well when agar was present in the medium, so he rooted the cultures in liquid medium using Heller bridges.

MS media has been used for the micropropagation of *P. pyrifolia* (Bhojwani et al., 1984); *P. communis*, cvs. Williams, Packham’s Triumph and Beurre Bosc (Shen and Mullins, 1984); *P. communis* cv. Conference (Baviera et al., 1989); *P. calleryana* (Beraardi et al., 1993); *P. bretecheideri*, cv. Jinhua (Wang et al., 1994); *P. syrica* (Shibli et al., 1997); compact clones of *P. communis* (Predieri and Govoni, 1998). *P. calleryana* (Racqual et al 2002a); *P. betulaefolia* (Racqual et al 2002b); *P. communis*, cv. Williams (Griegriandu et al., 2000).

Although in pear, most published micropropagation methods used Murashige and Skoog (MS) basal medium yet, some other media with slight modifications have also been used. Lepolivre (LP), Driver-Kuniyuki Walnut (DKW) and Woody Plant Media (WPM) have
improved shoot proliferation rates (Bell and Reed, 2002). Al Maarri et al., (1986) found Lepoivre medium (Quoirin et al., 1977) to be more efficient than MS medium for micropropagation of Passe-Crassane, in contrast to results from Baviera et al., (1989) on Conference. Likewise, Nedelcheva (1986) compared five mineral media (MS, Lepoivre, Schenk and Hildebrand, Gamborg, White) and found that the greatest number of rapidly growing buds of the cultivar Williams was produced on Lepoivre medium. The main features of Lepoivre medium are its low content of NH4$^+$ (4.9 mM) and relatively high content of Ca$^{++}$ (5.08 mM); the total ion content of Lepoivre medium is about ¾ of MS.

QL medium (Quoirin and Lepoivre, 1977) was the best for shoot proliferation of Highland pear (Grigoriadou et al, 2000). Freire et al (2002) reported the superiority of QL medium for the establishment of Pyrus communis, cv. Rocha uninodal cuttings. Yeo and Reed (1995) evaluated Cheng and Woody Plant Medium (WPM) containing a range of BA, NAA and IBA concentrations as potential media for shoot multiplication of three Pyrus rootstocks OPR 157, OPR 260 and CH X F 230. Cheng's medium with 8µM BA was the best for multiplication of all the genotypes, but auxin types varied. The lower concentration of major elements in WPM (Woody Plant Medium; Lloyd and McCown, 1980) was more suitable for micropropagation of CH X F (34, 51, 69, 87 and 230) pear rootstocks than the higher concentrations in MS medium (Nadcey, 1997). Similarly, WPM was more efficient than MS in inducing shoot proliferation in P. pashia (Dwivedi and Bist, 1997) and P. pyrifolia, cv. Gola (Dwivedi and Bist, 1999). Kadota et al., (2001) found that though Woody Plant Medium (WPM) produced highest number of shoots per explant in Japanese pear, yet it was at par with full and half strength MS medium. Viseur (1987) recommended the use of double-phase medium for micropropagation of P. communis, cvs. Durondeau, Conference, Doyenne du Comice and Professeur Molon based on a mineral composition which is intermediate between Lepoivre and MS media. According to Rooszban et al. (2002) woody plant medium (WPM) was the best medium for the growth of most of the genotypes of P. serotina (P. pyrifolia). Mehri Kamoun, et al. (2004) found that Cheng medium was the most effective for pear rootstocks CH X F 230, 217 and 69. Bahri Sahloul et al. (2005) found that MS medium with one-half NH4NO3 and KNO3 with BA at 4.4 µM, and Lepoivre with BA and IBA were the best for culture initiation in pear rootstocks (Old Home x Farmingdale, CH X F 69, 40 and 87). Previati et al. (2002) reported higher rooting percentage with MS medium supplemented with 3.0 mg IAA/l than with QL medium, which contained 2.0 mg IAA/l in P. communis rootstocks. Thakur (2004) found that in all the five genotypes of pear highest shoot proliferation rates were obtained with Woody Plant Medium (WPM), however, it was on a par with shoot proliferation rates on Murashige and Skoog medium (MS).

Plant material

Explant type: Any plant part, organ or tissue can be used as an explant. The healthy, young and soft explants (actively growing shoots) are generally more amenable for tissue culture than older woody tissues. Actively growing shoot tips are used for meristem, shoot tip and bud cultures because of their strong growth potential and low virus concentration (Hu and Wang, 1983). Starting material for establishment of pear in vitro culture consists mostly of shoot tips or single node explants from grafted plants grown in the greenhouse (Chevreau et al., 1992). Better results were obtained with explants taken from actively growing plants. However, dormant buds of the Japanese pear Koeui have also been used by Yotsuya et al., (1984). Shoot tips from the current season's
growth have been widely used as explants for in vitro propagation of pear. Shoot tips have been used to culture P. pyrifolia, cvs. Jinfeng and Zaofu (Zhao, 1982); P. pyrifolia (Bhojwani et al., 1984); P. pyrifolia, cvs. Kashi, Hua, Gani, Jiji, Yuji, Yang, Shinaui and Nijisseiki (Banno et al., 1989); P. pyrifolia cv. Gola (Dwivedi and Bist, 1999). P. communis, cv. Passe Crasanne (Al Maarri et al., 1986 and 1994); P. communis, cv. Conference (Baviera et al., 1989); P. communis, rootstocks OH x F 34, 51, 69, 87 and 230 (Nakaya, 1997); P. communis, compact clones (Predieri and Govoni, 1998); P. syricca (Shibli et al., 1997); and P. pashia (Dwivedi and Bist 1997). In pear, nodal segments have been used for establishing the cultures of P. communis, cvs. Williams, Bon Chretien, Packham’s Triumph and Beurre Bosc (Shen and Mullins, 1984); P. pyrifolia, cvs. Houtui, Hua, Nijisseiki, Shinaui (Bhojwani et al., 1984); P. calleryana cv. Bradford (Shimart and Harbage, 1989); P. bretscheri, cv. Jinhua (Wang et al., 1994); P. calleryana, OR 157, P. betulaefolia, OR 260 and P. communis, OH x F 230 (Yeo and Reed, 1995); P. communis, cv. Rocha (Freire et al., 2002) and P. pyrifolia, cvs. Houtui, Qianxi, Nijisseiki and P. communis, cvs. Packham’s Triumph and Red Bartlett (Dantas et al., 2002).


**Explant size**: The size of the explant determines the survival of the culture. In general, the larger the explant, the better the chance of survival. Hence, large explants such as shoot tips and buds should be selected for in vitro micropropagation instead of the minute meristems. However, when eradication of viral infection is one of the objectives, meristems of the smallest size, while still within the regenerable range, should be used (Hu and Wang, 1983). Explants ranging from 0.2 mm to 20 mm have been used for micropropagation of pear. Nicolodi and Pieber (1989) used 0.2-0.3 mm long meristems with up to three leaf primordia for the micropropagation of P. betulaefolia. Zhao (1982) cultured 0.5 mm shoot tips of P. pyrifolia, cvs. Jinfeng and Zaofu for their in vitro propagation. Banno et al. (1989) used shoot tips (<0.5 mm) for micropropagation of six Asian pear cultivars. Shibli et al., (1997) used shoot tips ranging from 0.5-0.7 mm in P. syricca. Meristem tips (0.5-1.5 mm) were used for micropropagation of four cultivars of P. communis. Bhojwani et al., (1984) used 1.0 mm shoot tips in P. pyrifolia. Ten cm long nodal segments were used by Shen and Mullins (1984) in P. communis. Wang et al., (1994) used 10-15 mm nodal segment for the micropropagation of P. bretscheri. Similarly, 10-15 mm long shoot tips were used by Dwivedi and Bist (1997, 1999) for in vitro clonal multiplication of P. pashia and P. pyrifolia, cv. Gola. Predieri and Govoni (1998) used 15-20 mm shoot tips for the micropropagation of compact clones of P. communis. Twenty long shoot tips were used as explants for in vitro shoot proliferation of P. communis, cv. Seckel (Singha, 1982). Yeo and Reed (1995) cultured single node explants for the micropropagation of three Pyrus rootstocks. Similarly, uninodeal explants were used in P. communis, cv. Rocha (Freire et al., 2002).

**Season of explant collection**: The success of shoot tip and bud culture is affected by the season during which explants are obtained. In plant species with a definite dormant period,
the best results are expected when the explants are at the end of the dormancy period (Hu and Wang, 1983). The best time for culture initiation is the beginning of intensive shoot growth just after bud break during spring (Hempel, 1991). According to Wang et al. (1994) maximum explant establishment occurred from November to February and it decreased rapidly during the period April to August in ‘Jinhua’ pear.

Explants were excised from actively growing shoots in *P. communis* (Lane 1979a) and *P. pyrifolia* (Banno et al., 1988 and 1989). Shibli et al., (1997) excised the explants of *P. syrica* from one year old dormant wood when the buds began to swell in early spring. Similarly, explants were collected during the spring season in *P. pashia* (Dwivedi and Bist, 1997); *P. pyrifolia* cv., *Gola* (Dwivedi and Bist, 1999); and *P. calleryana* (Stimart and Harbage, 1989).

**Surface sterilization of explant:** The woody plants are grown in soil for many years under ambient conditions and they are routinely infected with microorganisms both internally and externally which are often difficult to control in vitro. Therefore, explants need surface sterilization before culturing. Keeping the shoot tips in running water for an hour prior to a single surface sterilization has been found effective. This treatment also caused leaching of water soluble phenols and other growth inhibitors (Jones et al., 1979) and effectively reduced the infection (Hughes, 1981).

Lane (1979a) surface sterilized Bartlett pear with 2.5 per cent sodium hypochlorite for 15 minutes. Singh (1980) used 0.52 per cent sodium hypochlorite for 10 minutes for glasshouse grown ‘Seckel’ pear. Bharwani et al., (1984) sterilized the explants of *P. pyrifolia* with 0.6 per cent sodium hypochlorite for 30 minutes. In *P. communis*, Viseur (1987) sterilized the explants with 94 per cent calcium hypochlorite for 20 minutes, while, Baviera et al. (1989) used 0.7 per cent sodium hypochlorite solution in active chlorine for 20 minutes for sterilizing *P. communis*, cv. Conference.

Similarly, Bazzzardi et al., (1989) surface sterilized the seeds of open-pollinated *P. calleryana* by dipping, first in 70 per cent ethanol for a few seconds, then in sodium hypochlorite solution (0.18% available chlorine) for 25-30 minutes. Al Maarri et al., (1994) sterilized explants of *P. communis* by dipping them in 95 per cent ethanol for 30 seconds, then immersing them for 20 minutes in 10 per cent Domestos (a commercial preparation of sodium hypochlorite, with 7 per cent active chlorine).

Internal contamination also posed serious problems in woody plants and this problem has been overcome by adding 10 mg l⁻¹ benomyl or benlate to the culture medium.

**Growth regulators:** A balance between endogenous and exogenous growth regulators controls the initiation and development of shoots, roots, plantlets and callus. Skoog and Miller (1957) reported for the first time that the ratio of auxin to cytokinin determined the type and extent of organogenesis in tissue cultures. In general, cytokinins favour meristem proliferation, auxins induce callus formation and rooting and gibberellins induce stem elongation.

**Shoot multiplication:** In “axillary shoot proliferation”, cytokinins are utilized to overcome the apical dominance of shoots and to enhance the branching of lateral buds from leaf axils. In general, BA is the most effective cytokinin for stimulating axillary shoot proliferation, followed by kinetin and 2-isopentyladenine (2iP) (Hu and Wang, 1983).

Exogenous auxins do not promote axillary shoot proliferation, however, culture growth may improve by their presence. Too high a concentration of auxin may not only...
inhibit axillary bud branching (Hasegawa 1980), but also induce callus formation.

Gibberellins are not considered essential for in vitro culture and are very rarely used in tissue culture of higher plants. Its role is essentially for axillary bud elongation. BAP is the most frequently used cytokinin for pear micropropagation, at concentrations ranging from 4.4 to 22.2 µM (Chevreau et al., 1992). Lane (1979a) reported that 5 µM BAP was found to be effective for healthy shoot proliferation in P. communis. Addition of 0.3 µM GA, and 2.5 µM NAA with BAP was also effective for shoot elongation in P. communis, cv. Seckel (Singha, 1980). Zhao (1982) successfully proliferated Japanese pear cvs. Jinfeng and Zaoou, shoot tips pre-treated with GA, (50-200 mg l⁻¹) on MS media supplemented with 1 mg l⁻¹ BA + 10 mg l⁻¹ GA. Shen and Mullins (1984) found that the association of BAP (6 to 10 µM) with Zeatin (4.5 µM) and 2-i P (4.9 µM) was optimum for proliferation of the P. communis cultivars Williams, Packham’s Triumph and Beurre’ Bosc.

Japanese pear rootstock explants proliferated best in the medium supplemented with 1.0 mg l⁻¹ and 0.1 mg l⁻¹ IBA (Banno et al., 1988). Stimart and Harbage (1989) found that for P. calleryana, cv. Bradford medium with 2 µM BA and 0.5 µM IBA was best for optimum shoot proliferation and length. Baviera et al. (1989) found a positive correlation between BAP concentration and shoot multiplication rate. Liaw et al. (1992) obtained 5.8 and 9.7 shoots of P. serotina, cv. Hengshan and P. kwakamii, cv. Niauli, respectively within 30 days of transfer of established explants to MS medium containing BA (2 mg l⁻¹), IBA (0.2 mg l⁻¹), Kinetin (0.5 mg l⁻¹) and adenine sulphate (4 mg l⁻¹). The highest proliferation rate was attained at 1 mg l⁻¹ BAP, while the longest shoots were at 0.5 mg l⁻¹ BAP. NAA had no influence on shoot proliferation and length (Bernardi et al., 1993).

Yeo and Reed (1995) found that 8 µM BA and 0.5 µM IBA was the best growth regulator combination for shoot proliferation of GR 260 and GR x F 230 (P. calleryana). Nadsoy (1997) reported that low concentrations of auxins (0.05 mg IAA l⁻¹) and BA (0.5 mg l⁻¹) were more effective for shoot multiplication in hybrid pear rootstocks (GR x F 34, 51, 69, 87, 230), BA 29, pear seedlings and cultivars (Clapp’s Favourite and Bartlett). Increasing BA concentration to 1.5 and 2.0 mg l⁻¹ enhanced shoot proliferation significantly, while shoot height decreased in P. syriaca (Shibli et al., 1997). In P. pashia, Dwivedi and Bist (1997) found that the number of shoots per culture increased with increase in BA level up to 2 mg l⁻¹ and further, increase in the levels of BA reduced the number of shoots per culture. In P. communis cv. Bartlett, the highest number of shoots (3.87) were obtained with 2.4 mg BA l⁻¹. Predieri and Govoni (1998) micropropagated seven compact pear clones obtained through in vitro irradiation and two standard growth cultivars ‘Conference’ and ‘Doyenne d’ Hiver’. All compact clones, except clone F8, had the highest proliferation rates with the highest tested concentration of BAP (17.6 µM). This contrasted with the standard cultivar response. Conference had the highest shoot proliferation with 4.4 or 8.8 µM BA and Doyenne d’ Hiver with 4.4 µM BAP. Both standard cultivars had poor shoot multiplication with 17.6 µM BAP. In P. pyrifolia, cv. Gola, Dwivedi and Bist (1999) obtained maximum shoot multiplication (4.32) with a combination of BA (1.5 mg l⁻¹) and IBA (1.0 mg l⁻¹). The combination of 1 mg benzyladenine with 0.1 mg IBA/l for multiplication produced 2.5 new shoots with larger and well-expanded leaves in Pyrus communis cv. Koporecka, which were ideal for acclimatization (Sedlak and Paprstein, 2003). A concentration of 1 mg TDZ/litre caused abundant callus at the explant base and high hyperhydricity levels in the growing shoots. While studying the in vitro propagation
potential of five per genotypes, Thakur (2004) found that BA (2.0 mg l\(^{-1}\)) + IBA (0.5 mg l\(^{-1}\)) induced maximum shoot proliferation in wild pear (12.30 shoots/culture), kainth (7.79 shoots/culture) and shiara (5.24 shoots/culture). In Punjab Beauty and Patharnakh, the highest shoot proliferation rates of 6.81 and 5.33 shoots per culture, respectively were obtained with BA (1.5 mg l\(^{-1}\)) + IBA (0.5 mg l\(^{-1}\)).

**Rooting of microcuttings:** In case of woody species, in vitro rooting of microcuttings probably is the most difficult of the three stages to accomplish (Hu and Wang, 1983). The concentration of mineral salts and sugar is usually reduced to half or less of the concentration used for proliferation, cytokinin is excluded from the medium, and auxin concentration is often increased for rooting of micro shoots.

Most authors used MS medium, full strength or diluted, for rooting pear. Ochatt and Caso (1984) found liquid medium to be more efficient than agar-containing medium. Marino (1984) used short liquid pretreatment with IBA (1000 µM) followed by transfer to solid hormone free medium and obtained a good rooting percentage but the survival of plants during acclimatization was lower than that obtained after induction on solid medium containing auxin. Hirabayashi et al., (1987) found liquid IBA pretreatment (1000 µM), followed by transfer to sterile vermiculite plus MS medium, to be the more efficient technique for rooting of asian pear variety ‘Hosui’. Leite et al. (2000) while studying the effect of varying levels of sucrose concentration (0, 10, 20, 30 g l\(^{-1}\)) and light intensity on rooting of CHRF 97 pear rootstock found that increasing the light intensity from 2000 to 3000 lux increased the number of roots per plantlet by 25%. The best rooting response was obtained with sucrose at 20 g l\(^{-1}\).

The different Mg\(^{2+}\), Ca\(^{2+}\), K\(^{+}\), NH\(_4\)\(^{+}\) and NO\(_3\)\(^{-}\) ion content and rates had significant effects on the rooting of *P. sinii langensii* plantlets in vitro (Liu et al., 2004). The highest and lowest difference among rooting rate, and number of roots and root length per plantlet among the different macroelements was 53.13%, 5.43 and 12.67 cm, respectively. Thakur (2004) found that in wild pear, irrespective of the growth regulator concentrations significantly higher rooting was obtained on solid medium as compared to liquid medium. However, Kainth, Punjab Beauty, Patharnakh and shiara showed better rooting response on liquid medium.

NAA is the mostly used auxin for inducing rooting followed by IBA, IAA and 2,4-D (Hu and Wang, 1983). At higher auxin concentration callus is formed at the shoot base which inhibits normal root development (Lane 1979a). Another reason that too high an auxin level in rooting media is undesirable, is that after ‘root initiation phase’, the ‘root elongation’ phase is very sensitive to auxin concentration, and it is inhibited by high concentration of auxin (Thimann 1977).

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 and Roozban et al., 2002). In *P. communis* cv. Bartlett, Lane (1979a) observed 70 per cent root initiation with 10 µM NAA, whereas IBA performed better at a lower concentration (1.0 µM) IBA. Further, the roots induced by NAA were thicker and more prolific, whereas those induced by IBA were longer and fibrous. Singha (1980) also preferred NAA over IBA for inducing roots in *P. communis*, cv. Seckel as basal callus developed on the microcuttings with IBA even at lower concentrations whereas, callus developed with NAA only at higher concentrations. Viseur (1987) reported that in
**P. communis** rooting rates on rooting medium containing NAA (1.5 mg l⁻¹) varied from 30 to 90% with the cultivar. Shen and Mullins (1984) reported up to 80 per cent of microcuttings of **P. communis**, cv. Williams Bon Chretien (Bartlett), Baccam's Triumph and Beurre Bosc formed roots on MS medium supplemented with either NAA (10 µM) or IBA (10 µM) (Callus was formed at the base of the cuttings during the first week after inoculation.

While working with Japanese pear rootstock (Banno et al 1988), reported the best rooting with 1.0 mg l⁻¹ IBA. Whereas, 2.0 mg l⁻¹ IBA was the best for rooting of Japanese pear cultivars (Banno et al., 1989). In **P. communis**, cv. Conference, Baviera et al (1989) observed that NAA caused the formation of basal callus and, with IBA the percentage of rooting was good but the number of roots per plant was lower than IAA. They further reported that the plants with more and bigger leaves rooted better and with a greater number of roots per shoot. Beraldi et al., (1993) using radioactive IBA found that the differences in rooting ability between two pear cultivars: Conference (easy-to-root) and Doyenne’s Hiver (difficult-to-root) was due to differences in auxin uptake and metabolism by the microshoots.

Bartish et al., (1994) obtained almost 100 per cent rooting in **P. communis** on medium with 0.3 mg IBA per litre for 2-3 days and subsequent transfer to half strength MS medium without growth regulators. In **P. communis**, cvs. Passe-Crassane and Williams (Bartlett), Al Maarri et al., (1994) studied the factors affecting the in vitro and ex vitro root formation but, could not ascertain any notable differences, in the percentage of rooted cuttings with NAA or IBA. The best quality root formation was observed with 0.2 mg l⁻¹ of NAA.

Yeo and Reed (1995) reported that **P. communis**, OH X F 230 had the highest percentage (>80%) of rooting of the three rootstocks, and it rooted well with all IBA and NAA treatments. In case of five American-bred hybrid pear rootstocks (OH X F 34, 51, 69, 87 and 230) rooting was generally better on the medium containing 2 mg l⁻¹ IBA as compared to the medium containing 5 mg l⁻¹ IBA (Nadosy, 1997). Further, **P. betulaefolia**, OPR 260 showed highest rooting response (42.9%) with 10 µM IBA with one week dark treatment.

Shibli et al., (1997) observed that IBA, IAA and NAA induced in vitro rooting in wild pear (**P. syrica**) and a medium of 72 per cent rooting was achieved with 3.0 mg l⁻¹ IAA. Neither activated charcoal nor polyvinyl pyrrolidine influenced in vitro rooting and ex vitro rooting following auxin treatment was unsuccessful. Lian et al. (1992) obtained 60 and 70% rooting in **P. serotina**, cv. Hengshan and **P. kawakamii**, cv. Niarli, respectively on ½MS medium containing phloroglucinol (PG) plus auxin and kept in dark for 6 days, before transplanting to auxin-free ½ MS medium. Rooting in darkness was also required for pear rootstocks OHxF69, but not for OHxF40 and OHxF87 (Bahri Sahloul et al., 2005).

Stimart and Harbage (1989) failed to induce rooting in microcuttings of **P. calleryana**, cv. Bradford even after 2 years of subculture and intermittent attempts at rooting. Reed (1995) screened 49 Pyrus species and cultivars and one selection of *B. veitchii* for in vitro rooting response. Twenty eight accessions, mostly **P. communis** cultivars rooted the best (e.g 50%). Rooting percentage varied between 6 and 44 per cent in 13 accessions. However, **P. betulaefolia**, **P. calleryana**, **P. hondoensis**, **P. koehnei**, **P. pashia**, **P. pyrifolia**, **P. regelii**, **P. ussuriensis** and the *B. veitchii* selection failed to root. Rooshen et al., (2002) also failed to induce rooting in the micropropagated shoots of all the nine improved Asian pear (**P. serotina** [**P. pyrifolia**]) cultivars after two months on ½ MS medium supplemented with IBA. Thakur (2004) found that in wild pear the highest rooting (86.29%) was obtained on solid
medium fortified with NAA + IBA (0.25 mg l⁻¹ each) and phloroglucinol (160 mg l⁻¹). In Punjab Beauty and shiara the highest rooting percentages of 48.97 and 31.97, respectively were obtained on liquid medium fortified with NAA and IBA (0.50 mg l⁻¹ each). Kaith microshoots showed 76.00 per cent rooting with one week exposure to IBA (0.5 mg l⁻¹) and phloroglucinol (160 mg l⁻¹) in dark followed by transfer to ½ MS basal liquid medium for three weeks in fluorescent light. 58.70 per cent rooting was obtained in Patharnakh with similar treatment, except IBA (0.5 mg l⁻¹) was replaced with NAA (0.5 mg l⁻¹).

**Acclimatization/hardening of plantlets:** Under in vitro conditions plants are heterotrophic and have low rates of photosynthesis. This is due to low light intensity, low carbon dioxide concentration and inhibition of photosynthesis by high sugar concentration in the medium. Nevertheless, after transfer to ex vitro conditions, most micropropagated plants develop a functional photosynthetic apparatus. But, the increase in light intensity is not linearly translated in an increase in photosynthesis. In in vitro regenerated plantlets, the response of photosynthesis to light is similar to that of shade plants, characterized by low photosynthetic rates, low light compensation and saturation points. Deficiencies in chloroplast structure, namely grana development and at the biochemical level, the low Rubisco activity also contributed to a limited photosynthetic activity. The in vitro regenerated plantlets exposed to low light intensities acquire shade characteristics and when they are transferred to ex vitro conditions at higher light irradiance, light stress can occur including photo-inhibition or even photo-oxidation of chlorophyll, the later being revealed by chlorotic and dry spots appearing in the leaf blade. Nevertheless, some species tolerate higher irradiance, than those under in vitro conditions without a major stress. The control and optimization of light is, therefore, essential for successful acclimatization, to decrease the period of acclimatization, to increase the survival rate and for the proper development of new structures in leaves. Relative humidity only influences the survival of plantlets after their removal from culture vessels, prior to that time, the cultures are in closed vessels with stable high humidity. Transfer of plants from in vitro conditions to the greenhouse is still problematic for most of the species. When plants are removed from culture vessels they undergo rapid water loss leading to desiccation and death if not handled properly during acclimatization phase.

The success of transplanting and survival of plants greatly depends on the quality of roots. IBA-induced roots of *P. communis*, cv. Bartlett were of better quality than those on NAA medium (Lane, 1979a). In principle, a plant with only one root is capable to survive, nevertheless, greater number of roots per plant may compensate for non-functional or damaged roots (Nemeth, 1986). In pear, acclimatization has been accomplished in various substrates by progressively decreasing the relative humidity.

**CONCLUSION**

Tremendous work has been done all over the world on micropropagation of pear. Efficient in vitro clonal propagation protocols have been developed for different pear rootstocks and varieties. In European countries, micropropagation has been utilized for large scale multiplication of commercial rootstocks (OH x F rootstocks) and cultivars belonging to *P. communis*. But, in India the technique is not being commercially exploited due to high cost involved, comparatively poorer ex vitro survival of plants and stagnation in the expansion of pear industry. The different in vitro culture techniques viz. utilization of somaclonal variations, in vitro mutagenesis and genetic transformation can be used to evolve new genotypes suitable for cultivation in tropics and subtropics so as to increase the acreage, production and quality of pear.
REFERENCES


