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BRIEF NOTE

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In vitro micro propagation of "sand pear" *Pyrus pyrifolia* (Burm. f.) Nakai

Abstract This study was undertaken to standardize protocols for *in vitro* explant establishment, shoot multiplication and *in vitro* rooting for *Pyrus pyrifolia*, growing in the Kashmir valley. The explant used was nodal cuttings of 2–3cm size with one or two axillary buds. The highest explant establishment (over 90%) was observed during spring while the lowest (60%) explant establishment frequency was observed during winter season. The shoots sprouted from the axillary buds were excised and subcultured on MS basal medium. The highest shoot multiplication rate (130 shoots from a single nodal cutting) was obtained on MS medium supplemented with BAP 2.2 mg·L^{-1} and kinetin 1 mg·L^{-1} after four weeks. The shoots were inoculated for rooting in root induction medium of half strength MS salts supplemented with plant growth regulators like IBA, IAA, NAA, and 2, 4-D. However, the best rooting response (8.50 roots per shoot) was obtained in combination of hormones (IBA + NAA) 0.25 mg·L^{-1} each. The rooted micro shoots were acclimatized under decreasing humidity regime (95% RH to 75% RH over a period of 5±1 weeks). The highest *ex vitro* plantlet survival percentage (56.50%) of *Pyrus pyrifolia* was observed on sand + soil (1:1 v/v).

Keywords *Pyrus pyrifolia*, micro propagation, shoot multiplication, nodal cuttings, Murashige and Skoog

1 Introduction

Pear is an important fruit of temperate zones, whose world wide production is only exceeded by apple. The cultivation of large numbers of trees through conventional methods results in higher initial costs. The use of tissue culture techniques especially micro-propagation is a potential method for the production of self rooted clones of pear cultivars. This eliminates the expenses involved in budding and grafting.

Pyrus pyrifolia (Burm. f.) Nakai "sand pear" is an important fruit in the Kashmir valley. It has a long shelf life and nice fragrance, thus fetching more revenue than other pear varieties. To boost its production, propagation with shortened life cycle and at cheaper rates is possible through micro propagation.

2 Materials and methods

The explants were excised from 15- to 20- year old trees of *Pyrus pyrifolia*. These nodal segments of 2–3 cm with one or two axillary buds were washed under tap water for 15 minutes. They were further washed with liquid detergent labolene (2%) and surfactant tween-20(1%) for 10 min. These explants were surface sterilized with 0.1% mercuric chloride solution for 6 min. Finally, these were washed with autoclaved double distilled water to remove the traces of mercuric chloride and inoculated in culture flasks of 100 mL capacity under laminar flow hood.

In the entire cultures, Murashige and Skoog (MS) basal medium was used. The medium was supplemented with different concentrations and combinations of auxins and cytokinins. The pH of the medium was adjusted to 5.8 with 0.1 N NaOH/HCl. The culture vials with media were autoclaved at 103442.35938 Pa pressure and 121°C temperature for 15 min. The inoculations were performed under laminar airflow hood with all necessary precautions. After inoculations, the cultures were transferred to a culture room for exposure to controlled conditions (16/8 h photoperiod, 24±2°C temperature, light intensity of 5000 lx and relative humidity of 80%).

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3 Results and discussion

Micro propagation protocols have been developed for several important rosaceous fruit crops such as apple (Cheng, 1978; James and Thurbon, 1979; Jones 1979; Sriskandarajah and Mullins, 1981; Sriskandarajah et al., 1982), various species of *Prunus* (Tabachnik and Kester, 1977; Jones and Hopgood, 1979) and pears (Singha, 1980; Poudyal and Zhang 2008).

The results in this paper include the phases of initiation, multiplication and rooting of *Pyrus pyrifolia*. The nodal explants with one or two axillary buds were inoculated on Murashige and Skoog (1962) medium supplemented with BAP 2 mg·L^{-1}(Fig. 1). The buds sprouted within 18±2 d. However, variation in sprouting percentage was observed in spring and autumn buds. Spring buds sprouted at the rate of over 90% while autumn buds sprouted at the rate of 60% only. The difference in sprouting percentage has earlier been reported in wild pear (Thakur and Kanwar, 2008a). The axillary shoots (15–20 mm) were excised and sub cultured on MS medium supplemented with various hormonal combinations. However, the best results (5.50 shoots/explant) were obtained at hormonal combination of BAP 2.2 mg·L^{-1}, IBA 2.0 mg·L^{-1} and kinetin 1 mg·L^{-1} (Table 1, Fig. 2). The synergism of two cytokinins has earlier been reported in apple (Dalal et al., 2006). The continuous sub culturing was done to multiply the shoots. After the third subculture, the number of shoots raised from single explants was 130 (Table 2, Fig. 3).

The micro shoots (20–30 mm) were inoculated for rooting in rooting media containing half strength MS salts supplemented with different auxins. However, the best rooting response (8.50 roots/shoot) was obtained on hormonal combination of (IBA + NAA) 0.25 mg·L^{-1}

Fig. 1 Sprouting of nodal cuttings

Fig. 2 Formation of multiple shoots after the 1st inoculation

Table 1 Effect of different hormonal combinations on shoot multiplication of *Pyrus pyrifolia*

plant growth regulator/(mg·L^{-1}) BAP + IBA + Kn	buds sprouted/%	shoot number±*SD*	shoot length (mm)±*SD*
MS basal	0.0	0.00±0.00	0.00±0.00
0.5 + 0 + 0	10.0	1.2±0.34	10.0±0.32
1.0 + 0.5 + 0	20.0	1.5±0.42	20.2±2.9
2.0 + 1.0 + 0	60.0	2.5±0.45	23.5±1.7
2.2 + 2.0 + 1.0	90.0	5.5±0.16	25.5±4.3
2.2 + 2.0 + 2.0	80.0	3.5±0.32	20.2±3.2
2.2 + 2.2 + 2.0	60.0	3.2±0.13	18.1±2.2

Note: Data scored at the end of 5±1 weeks and 10 replicates were taken in each treatment.

Table 2 Shoot multiplication and elongation of *Pyrus pyrifolia* in repeated subcultures

subculture	shoot number±*SD*	shoot length (mm)±*SD*
1st	4.00±0.31	23.5±4.8
2nd	25.6±9.2	24.6±5.3
3rd	130.5±10.2	25.5±4.5

Note: Data scored at the end of 5±1 weeks and 10 replicates were taken in each treatment.

Table 3 Effect of IBA and NAA on root regeneration in *in vitro* raised shoots of *Pyrus pyrifolia* on half strength MS medium

plant growth regulator/ ($mg \cdot L^{-1}$) IBA + NAA	response/%	root number±*SD*	root length (mm)±*SD*
0.1 + 0.0	20.0	0.00±0.00	0.00±0.00
0.2 + 0.2	25.0	2.0±0.42	7.0±0.2
0.25 + 0.25	70.0	8.5±1.40	20.5±4.0
0.5 + 0.5	65.0	6.3±2.1	23.2±3.6
1.0 + 1.0	50.0	5.2±1.2	15.5±4.5
2.0 + 0	30.0	3.2±2.5	18.4±5.1
4.0 + 0	25.0	3.1±2.0	15.2±4.3

Note: Data scored at the end of 5±1 weeks and 10 replicates were taken in each treatment.

Fig. 3 Formation of multiple shoots after the 3rd subculture

Fig. 4 Rooted shoots

each (Table 3, Fig. 4).

The shoots were exposed to this medium for 8±3 d and then transferred to MS full strength hormone free medium. The combination of auxins for rooting has been reported earlier (Thakur and Kanwar, 2008 b). The rooted plantlets were transplanted in polyvinyl cups containing pre-autoclaved sand and garden soil (1∶1 v/v) moistened with 1/4th strength MS salt solution. The plantlets were housed in a shaded zero energy polythene house maintaining gradually decreasing humidity regime (95% RH to (70±5)% RH) over a period of 5±1 weeks, which resulted in 56.50% survival.

4 Conclusion

The *in vitro* propagation protocol was developed for *Pyrus pyrifolia* using nodal segments. The explants used, i.e.,

nodal segments showed seasonal difference in sprouting. The spring buds sprouted at the rate of 90% while autumn/ winter buds sprouted at the rate of 60% only. The highest shoot proliferation was obtained by using two cytokinins and one auxin while the rooting took place at the half strength MS media supplemented with two auxins.

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