The Pharma Innovation Journal 2019; 8(4): 730-732

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(4): 730-732

www.thepharmajournal.com
Received: 21-02-2019
Accepted: 22-03-2019

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Establishment of explant type in different seasons and effect of plant growth regulator combinations on the *in vitro* multiplication of shoots of *Pyrus pyrifolia*

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Abstract
Pyrus pyrifolia is an important pome fruit of family *rosaceae.* The development of *in vitro* micro-propagation protocol is the need of hour for each and every plant. In this backdrop the stem cuttings of the *P. pyrifolia* were induced to develop shoots in Murashige and Skoog medium supplemented with different combinations of hormones in different concentrations. The optimum results are obtained for establishment of nodal cuttings during spring season while the least results are obtained for shoot tips during winter season. The established shoots are inoculated for multiplication and the optimum results of multiplication are obtained with using two cytokinins and one auxin in the combination of 2 mgL^{-1} BAP +2 mgL^{-1} IBA + 1mgL^{-1} Kn.

Keywords: *Pyrus pyrifolia*, Micro-propagation, Nodal cuttings, Cytokinins, Auxin

Introduction
Pyrus pyrifolia (Burm. f.) Nakai "Sand pear" is an important fruit of Kashmir valley. It has long shelf life, nice fragrance and fetches more revenue than other varieties. In order to boost its production, micro-propagation is a potential method for production of self-rooted clones of pear plants. This eliminates the expenses involved in budding, grafting and shortens its life cycle.

Materials and Methods
The explants were excised from 15 to 20 year old trees of *P. 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 then 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 (1962) [1]. (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 15 lbs 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 hour photoperiod, 24 ± 2°C temperature, light intensity of 5000 lx and relative humidity of 80%).

Results and Discussion
In vitro micro-propagation is an important method for the large scale development of clonal plantlets. 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) [2, 3, 6, 4, 5], and pears (Singha, 1980; Poudyal and Zhang, 2008) [11, 7].
The results in this paper include the phases of initiation, establishment and multiplication of shoots of *P. pyrifolia*. The nodal explants with one or two axillary buds were inoculated on MS medium. The buds sprouted within 18±2 d. However, variation in sprouting percentage was observed in spring, summer, autumn and winter buds.

Spring buds sprouted at the rate of over 80% while winter buds sprouted at the rate of 56% only (Table 1, Fig. 1 and Fig. 2). The difference in sprouting percentage has earlier been reported in wild pear (Thakur and Kanwar, 2008a; Thakur and Kanwar, 2008b) [8, 9].

Table 1: Effect of explant type and season of explant collection on the explant establishment of *Pyrus pyrifoli* on M S medium.

Season	Explant establishment %age	
	Shoot tip	Nodal segment
Spring	62.63	79.03
Summer	61.14	69.44
Autumn	60.38	67.31
Winter	56.70	70.27
LSD = 0.05%	1.71393	4.81997

Note: Data scored at the end of 4±1 weeks; 5 replicates per treatment.

Fig 1: Sprouting of buds from nodal cuttings inoculated in MS medium during spring season.

Fig 2: Sprouting of buds from nodal cuttings inoculated in MS mediu during winter season

The axillary shoots (15-20 mm) were excised and sub cultured on MS medium supplemented with various hormonal combinations. However, the best results (10.2 shoots/explant) were obtained at hormonal combination of BAP 2 mg·L^{-1}, IBA 2.0 mg·L^{1} and kinetin 1mg·L^{-1} (Table 2; Fig. 3 and Fig. 4). The synergism of two cytokinins has earlier been reported in apple (Dalal *et al*., 2006) [10]. 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: Interaction effect of different hormonal combinations on *in vitro* shoot multiplication of *Pyrus pyrifolia* using M.S medium supplemented with different combinations of plant growth regulators.

MS+Kn (mgL^{-1})	No. of shoots per explant	Average length of shoot (cm)	%age of cutting
2.0	1.6	1.52	80
3.0	2.4	2.30	90
4.0	2.2	1.74	80
MS+BAP+IAA			
2.0+0.5	2.2	1.92	30
2.0+1.0	2.6	2.22	40
2.0+2.0	2.4	2.14	40
MS+BAP+IBA			
1.0+2.0	3.2	3.18	80
2.0+2.0	3.6	3.56	90
2.0+2.5	3.4	3.42	90
MS+BAP+NAA			
1.0+1.0	1.6	2.3	60
2.0+1.0	2.2	2.7	70
0.5+2.0	1.7	2.1	40
MS+IBA+Kn			
1.0+2.0	3.4	3.26	90
2.0+2.0	3.6	3.46	90

1.0+2.5	3.2	3.58	80
MS+NAA+Kn			
1.0+1.0	2.6	1.66	40
2.0+1.0	2.8	2.66	40
2.5+1.0	2.6	1.88	30
MS+BAP+IBA+Kn			
1.0+2.0+1.0	9.0	3.86	90
2.0+2.0+1.0	10.2	3.9	90
0.5+2.0+2.0	8.4	3.54	90

Note: Data scored at the end of 4±1 weeks. 10 replicates per treatment.

Fig 3: Multiplication of shoots of P. pyrifolia on MS medium supplemented with 2mgL^{-1} IBA+1mgL^{-1} Kn IBA

Fig 4: Multiplication of shoots of P. pyrifolia on MS medium supplemented with 2mgL^{-1} BAP + 2mgL^{-1} + 1mgL $^{-1}$ Kn

Conclusions

In vitro shoot micro propagation protocol was developed for *P. pyrifolia* using nodal segments and shoot tips during four different seasons of the year. The spring buds sprouted at the rate of 80% while winter buds sprouted at the rate of 56% only. The sprouted shoots where inoculated for shoot multiplication. The highest rate of shoot proliferation was obtained by using two cytokinins and one auxin.

Acknowledgments

The authors thank the Council for Scientific and Industrial Research (CSIR) for full time financial assistance for this research program.

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