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In vitro Propagation of Kainth (*Pyrus pashia*) Using Explants from Forced Cutting

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Abstract

This study was carried out in Tissue culture laboratory, Department of Fruit Science, Punjab Agricultural University, Ludhiana during 2011-13. The effect of various media {1/2 MS (M_1), MS (M_2) and WPM (M_3)} and growth regulators (BAP, IBA and NAA) on establishment, proliferation and rooting was studied. Maximum establishment (63.60%) was obtained on M_2 containing BAP (1.5 mgl^{-1}) and IBA (0.25 mgl^{-1}). Maximum proliferated cultures (95.30%) and shoots per explant were obtained using M_3 medium fortified with BAP (3.0 mgl^{-1}). However, shoots of maximum length (42.97 mm) were obtained in M_3 medium containing BAP (0.0 mgl-1) i.e control. Rooting (%), roots per explant and root length was found to be influenced by type of medium and growth regulator fortification. Rooting (%) was maximum (13.34%) was observed in M_1 medium supplemented with IBA (0.1 mgl^{-1}). However, NAA (1.0 mgl^{-1}) induced rooting of 29.61 per cent using M_1 medium. Maximum roots per explant were obtained using M_1 medium supplemented with IBA (1.0 mgl^{-1}). NAA (1.0 mgl^{-1}) induced highest roots per explant i.e 3.40 using M_1 medium. However, M_3 supplemented with IBA (0.1 mgl^{-1}) resulted in maximum root length of 31.15 mm. NAA (0.1 mgl^{-1}) resulted in maximum root length of 22.97 mm using M_1 medium.

Keywords: Media; Establishment; Proliferation; Rooting; Fortification

Introduction

The use of tissue culture for fruit and nut tree species have increased substantially since the early 1970s and virtually all fruit tree species have been micropropagated with various degrees of success. Seedling rootstocks are not uniform in growth and productivity [1]. Therefore, vegetative propagation methods like cutting and stooling are used to multiply pear rootstocks. Micropropagation has shown promises for rapid and large scale clonal multiplication of disease free planting material throughout the year. *In vitro* propagation has been reported in several pear rootstocks viz. P *betulaefolia* L. [2], Wild pear [3]; OPR 157, OPR 260 and OH × F 230 [4]; P *calleryana* [5] and quince [6], Pyrodwarf [7] and *Pyrus communis* L. rootstock [8].

Materials and Methods

For forcing, dormant cuttings of Kainth of 15-20 cm in length (10-15 mm diameter) were collected and stored at 4±3°C in polythene bags. After subjecting the requisite chilling units, the cuttings were withdrawn and basal ends were re-cut by about 1 cm and placed in polythene bags containing rooting medium, covering about 5 cm of basal portion of cuttings. The cuttings were incubated in growth chamber at 23±1°C under 16 hours photoperiod with light intensity of 3000 lux. Shoots put forth by the sprouted buds served as explant source for in vitro propagation (Figure 1).

The basal media used in the study were Murashige and Skoog's medium (MS), Murashige and Skoog's medium with half strength of macro and micronutrients (1/2 MS) and Woody Plant Medium (WPM).

Figure 1: Forced cuttings of Kainth.

Already prepared MS medium (PT021) and WPM (PT026) purchased from Hi Media Pvt. Limited was used to prepare basal media i.e. M_1 (half strength MS), M_2 (full strength MS) and M_3 (Woody Plant Medium). Sterilized explants were inoculated in test tubes/glass jars containing autoclaved media for establishment. Different media (½ MS, MS and WPM) fortified with different combinations of 6-benzylaminopurine (BAP) {(0.5-3.0 mgl^{-1}) and IBA (0.01-2.0 mgl^{-1})} were used. The data were recorded on necrotic cultures (%), explant establishment (%) after 3 weeks of inoculation.

Established explants were transferred to shoot proliferation media. Various shoot proliferation media i.e. ½ MS, MS and WPM were used, fortified with various concentrations of BAP i.e. 0.5-5.0 mgl-1. The

optimum media and concentration of BAP for shoot proliferation was standardized. Observations on proliferated cultures (%), number of shoots/explant and average length of shoot (mm) was recorded after third subculture and the culture duration were four weeks each.

After allowing shoots to multiply on shoot proliferation medium, individual shoots were separated (20 mm long) and transferred to root regeneration medium. Different types of media i.e. ½ MS, MS and WPM containing various combinations of IBA (0.1-2.0 mgl^{-1}) and NAA (0.1-2.0 mgl^{-1}) were used. Observations on rooting (%), number of roots/ explant and average length of roots (mm) were recorded after four weeks.

Results and Discussion

The results of present investigation are described under appropriate heads supplemented with tables and plates.

Effect of medium supplemented with growth regulators on explant establishment

Data in Table 1 reveal that both media and growth regulator had significant effect on establishment (%). Highest establishment of 23.55 per cent was achieved by using M_3 medium, which was significantly higher as compared to M_2 and M_1. The data clearly shows that establishment percentage was highest using BAP (1.5 mgl^{-1}) and IBA (0.25 mgl^{-1}) and this combination differed significantly from all other combinations. Interaction studies between medium and growth regulators revealed that the highest explant establishment of 52.80 per cent was achieved from M_2 medium fortified with BAP (1.5 mgl^{-1}) and IBA (0.25 mgl^{-1}) (Figure 2).

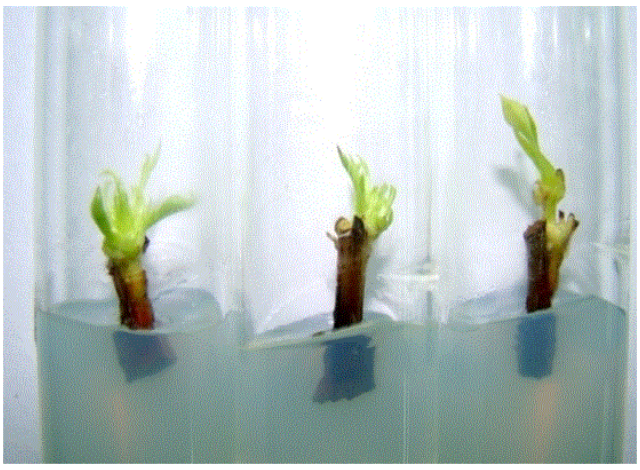

Figure 2: Explant establishment in MS medium fortified with BAP (1.5 mgl^{-1}) + IBA(0.25 mgl^{-1})

M1 medium fortified with BAP (0.5 mgl^{-1}) and IBA (0.5 mgl^{-1}) resulted in the lowest establishment (%). These results are in conformity with earlier observation made by De Paoli, [9], who reported Murashige and Skoog medium as the best medium for culture initiation resulting in the highest establishment (%) with least necrotic cultures. Variations in per cent establishment of explants with different doses of auxin and cytokinin during micropropagation are in conformity with the reports given by Fan and Jiang [10] in apple; [11] in *Carica papaya*; [12] in pear; [13] in *Citrus acida*; [14] in pineapple and [15] in sweet cherry. Different media have been tried earlier for establishment of plant species by various workers and reported varied results in terms of establishment percentage. Peer et al. [16] reported better results in terms of establishment percentage in sweet cherry cv. Bigarreau Noir Grossa using Murashige and Skoog medium over Driver and Kuniyuli medium, Woody Plant Medium and Knop's macro and MS micro-organics medium independent of growth regulators concentration. In vitro effects of growth regulator on overall establishment of explant has been reported to be influenced by growth medium composition, growing conditions and genotype [17,18].

Growth regulator combination (mgl^{-1})	Media			Mean
	M_1(1/2 MS)	M_2(MS)	M_3(WPM)	
BAP(0.5)+IBA(0.01)	21.32	25.05	16.61	20.99
BAP(0.5)+IBA(0.25)	0.40	9.11	25.04	11.52
BAP(0.5)+IBA(0.5)	0.05	0.21	12.47	4.24
BAP(1.0)+IBA(0.01)	11.12	26.68	50.14	29.31
BAP(1.0)+IBA(0.25)	8.36	14.19	41.49	21.35
BAP(1.0)+IBA(0.5)	5.29	11.29	11.72	9.43
BAP(1.5)+IBA(0.01)	18.82	26.36	31.27	25.48
BAP(1.5)+IBA(0.25)	11.06	52.80	27.30	30.39
BAP(1.5)+IBA(0.5)	5.09	18.71	14.30	12.70
Control	5.67	10.40	5.12	7.063
Mean	8.72	19.48	23.55	
C.D($p \leq 0.05$)	Media (A)=0.881, GR's (B)= 1.608, A×B=2.786			

Table 1: Effect of media type and growth regulators (mg/l) on explant establishment (%).

Shoot proliferation

Established explants were transferred to shoot proliferation media i.e. M_1, M_2 and M3 containing different levels BAP. Data regarding shoot proliferation was obtained after two sub culturing as shoot growth was very slow initially irrespective of media used and growth regulator level.

From the perusal of (Table 2) it is evident that proliferated culture (%) during proliferation stage is certainly affected by type of media and BAP concentration individually as well as in interactive fashion. With regard to media, irrespective of BAP concentration, maximum proliferated culture (72.13%) was obtained in M_3, which is significantly higher than proliferated culture (%) obtained in M_2 and M_1. Maximum proliferated cultures (91.13%) resulted by using BAP at 3.0 mgl^{-1}, which is statistically at par with proliferated culture (%) obtained at BAP (1.5 mgl^{-1}). Regarding interaction between type of media and BAP concentration, maximum proliferated cultures (95.30%) were obtained by using M_3 fortified with BAP (3.0 mgl^{-1}) (Figure 3).

Growth regulator (mgl^{-1})	Media			Mean
	M_1(1/2 MS)	M_2(MS)	M_3(WPM)	
BAP(0.5)	60.03	71.43	66.65	66.04
BAP(1.5)	84.59	92.51	92.35	89.82
BAP(3.0)	86.44	91.64	95.30	91.13
Control	13.93	25.49	34.23	24.55
Mean	61.25	70.27	72.13	
C.D($p \leq 0.05$)	Media (A)= 1.551, BAP (B)= 1.790, A×B=3.101			

Table 2: Effect of media type and growth regulator level (mgl^{-1}) on proliferated cultures (%).

Figure 3: Shoot proliferation in WPM fortified with BAP (3.0 mgl^{-1}).

Data in (Table 3) clearly shows that with increase in level of BAP, there is increase in number of shoots produced per explant irrespective of media used. Maximum number of shoots per explant was observed in M_3 (4.39) which was followed by M_2 (3.61) and M_1 (2.63). All media differ significantly in terms of production of number of shoots per explant. The highest number of shoots per explant i.e. 4.78 was obtained with BAP (3.0 mgl^{-1}) and it was followed by 1.5 mgl^{-1} BAP (4.43). Interaction effect revealed that there is significant interaction between media and growth regulator concentration on number of shoots produced per explant in Kainth. M_3 medium fortified with BAP (3.0 mgl^{-1}) resulted in maximum number of shoots per explant (6.28) followed by 5.75 in M_3 supplemented with BAP (1.5 mgl^{-1}).

Shoots of most desirable length (40.86 mm) were obtained on M_3 medium followed by 38.86 mm on M_1 medium (Table 4). From the perusal of Table 5, shoot length clearly decreases with increase in level of BAP and shoots of maximum length were obtained when no growth regulator was added to medium i.e. control. Average shoot length produced at 0.5 mgl^{-1} BAP (41.79 mm) and control (42.19) were statistically at par but differ significantly from average shoot length produced at 1.5 mgl^{-1} BAP (37.20 mm) and 3.0 mgl^{-1} BAP (33.22 mm). There was no significant interaction between type of media and growth regulator level on average length of shoots although maximum length of shoots was obtained when basal M_3 medium i.e. control was used during shoot proliferation.

Growth regulator (mgl^{-1})	Media			Mean
	M_1(1/2 MS)	M_2(MS)	M_3(WPM)	
BAP(0.5)	2.76	2.59	2.91	2.75
BAP(1.5)	2.92	4.61	5.75	4.43
BAP(3.0)	3.15	4.92	6.28	4.78
Control	1.68	2.33	2.62	2.21
Mean	2.63	3.61	4.39	
C.D($p \leq 0.05$)	Media (A)=0.141, BAP (B)=0.163, A×B=0.282			

Table 3: Effect of media type and growth regulator level (mgl^{-1}) on number of shoots.

Growth regulator (mgl^{-1})	Media			Mean
	M_1(1/2 MS)	M_2(MS)	M_3(WPM)	
BAP(0.5)	42.10	40.31	42.97	41.79
BAP(1.5)	39.05	33.53	39.01	37.20
BAP(3.0)	31.02	31.37	37.26	33.22
Control	43.27	39.11	44.20	42.19
Mean	38.86	36.08	40.86	
C.D($p \leq 0.05$)	Media (A)=1.924, BAP (B)=2.222, A×B=NS			

Table 4: Effect of media type and growth regulator level (mgl^{-1}) on av. length of shoots (mm).

These findings are in conformity with those of in *P. pyrifolia* cv. in Japanese pear cultivar Hosui, who reported superiority of WPM over other media with respect to proliferation rate [19,20]. BAP level was found to effect significantly per cent proliferation, shoots per explant and shoot length and these results are in conformity with studies reported by [19]; [22]; Karimpour et al. [18]; Hassanen and Gabr [2]; Ruzic et al. [7]; Isikalan et al. [23] and Soni et al. [24]. Hu and Wang [25] reported that cytokinins, especially BAP stimulated axillary bud development but at higher concentration shoot elongation was suppressed. Similarly, higher number of shoots per explant during proliferation stage on M2 as compared to M1 has been reported by Hassan [26] in Le Conte pear, [27] in Bartlett pear and [28] on figure due to higher nutrient concentration.

In vitro rooting

The in vitro regenerated shoots during shoot proliferation were transferred to various rooting media supplemented with different levels of IBA and NAA. Data from (Table 5) reveal that the highest rooting of 5.22 per cent was observed on M_1 medium, which is significantly different from rooting percentage observed on M_2 and M_3. Irrespective of media, IBA (0.1 mgl^{-1}) induced higher rooting

(1.58%) as compared to IBA (1.0 mgl^{-1}). The interaction effect of treatment combination of M_1 fortified with IBA (0.1 mgl^{-1}) resulted in best rooting response (13.34%) (Figure 4), when compared to M_3 fortified with IBA (0.1 mgl^{-1}).

Growth regulator (mgl^{-1})	Media			Mean
	M_1(1/2 MS)	M_2(MS)	M_3(WPM)	
IBA (0.1)	13.34	9.04	9.36	10.58
IBA (1.0)	7.55	0.00	0.00	2.52
IBA (2.0)	0.00	0.00	0.00	0.00
Control	0.00	0.00	0.00	0.00
Mean	5.22	2.26	2.34	
C.D($p \leq 0.05$)	Media (A)=0.705, IBA(B)=0.815, A×B=1.411			

Table 5: Effect of media type and IBA level (mgl^{-1}) on rooting (%).

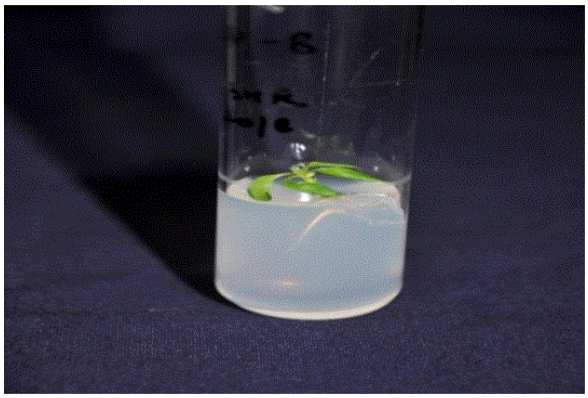

Figure 4: Rooting using ½ MS fortified with IBA (0.1 mgl^{-1}).

Data in (Table 6) clearly reveals that the highest number of roots per explant (1.25) was produced in M_1, which is significantly higher than those obtained on M_2 and M_3.

Growth regulator (mgl^{-1})	Media			Mean
	M_1(1/2 MS)	M_2(MS)	M_3(WPM)	
IBA (0.1)	2.20	2.01	1.81	2.01
IBA (1.0)	2.79	0.00	0.00	0.93
IBA (2.0)	0.00	0.00	0.00	0.00
Control	0.00	0.00	0.00	0.00
Mean	1.25	0.50	0.45	
C.D($p \leq 0.05$)	Media (A)=0.057, IBA(B)= .066, A×B=.114			

Table 6: Effect of media type and IBA level (mgl^{-1}) on number of roots per explant.

Maximum number of roots per explant (2.01) was obtained by using IBA at 0.1 mgl^{-1} irrespective of media used. M_1 medium fortified with IBA (1.0 mgl^{-1}) resulted in significantly higher number of roots per explant (2.79) than what was obtained in M_1 medium fortified with IBA (0.1 mgl^{-1}).

Perusal of data in (Table 7) clearly reveals that there is significant effect of rooting media and IBA levels on average length of roots. Irrespective of IBA levels, M_1 induced average root length of 11.00 mm as compared to 7.79 mm on M3 and 7.04 mm on M_2 medium. While as IBA at 0.1 mgl^{-1} resulted in maximum average root length of 27.41 mm, which is significantly higher than 7.03 mm observed at IBA (1.0 mgl^{-1}). Interaction effect revealed that roots of maximum length were produced in M_3 medium fortified with IBA (0.1 mgl^{-1}) having average length of 31.15 mm, which is significantly higher than other treatment combinations.

Growth regulator (mgl^{-1})	Media			Mean
	M_1(1/2 MS)	M_2(MS)	M_3(WPM)	
IBA (0.1)	22.90	28.17	31.15	27.41
IBA (1.0)	21.10	0.00	0.00	7.03
IBA (2.0)	0.00	0.00	0.00	0.00
Control	0.00	0.00	0.00	0.00
Mean	11.00	7.04	7.79	
C.D($p \leq 0.05$)	Media (A)=1.226, IBA(B)=1.416, A×B=2.452			

Table 7: Effect of media type and IBA level (mgl^{-1}) on average length of roots (mm).

As compared to IBA, NAA induced higher rooting irrespective of type of rooting media used (Table 8). Data from Table 9 clearly reveals that M_1 induced the highest rooting (13.49%) in Kainth. NAA at 1.0 mgl^{-1} resulted in maximum rooting (24.83%), which is significantly higher than 22.35 per cent obtained at NAA (0.1 mgl^{-1}). A treatment combination of M_1 with NAA (1.0 mgl^{-1}) resulted maximum rooting of 29.61 per cent (Figure 5).

Growth regulator (mgl^{-1})	Media			Mean
	M_1(1/2 MS)	M_2(MS)	M_3(WPM)	
NAA (0.1)	24.35	23.41	19.30	22.35
NAA (1.0)	29.61	24.94	19.95	24.83
NAA (2.0)	0.00	0.00	0.00	0.00
Control	0.00	0.00	0.00	0.00
Mean	13.49	12.09	9.81	
C.D($p \leq 0.05$)	Media (A)=1.006, NAA(B)=1.162, A×B=2.012			

Table 8: Effect of media type and NAA level (mgl^{-1}) on rooting (%).

With regard to effect on number of roots per explant using different types of rooting media fortified with various levels of NAA showed that M_1 resulted in maximum number of roots per explant. From the perusal of data in (Table 9), significantly more number of roots per explant (3.07) were observed using NAA (1.0 mgl^{-1}), irrespective of media. M_1 fortified with NAA (1.0 mgl^{-1}) resulted in the highest number of roots per explant (3.40).

Figure 5: Rooting using ½ MS fortified with NAA (1.0 mgl^{-1}).

Growth regulator (mgl^{-1})	Media			Mean
	M_1(1/2 MS)	M_2(MS)	M_3(WPM)	
NAA (0.1)	3.02	2.78	2.43	2.74
NAA (1.0)	3.40	3.22	2.60	3.07
NAA (2.0)	0.00	0.00	0.00	0.00
Control	0.00	0.00	0.00	0.00
Mean	1.61	1.50	1.26	
C.D($p \leq 0.05$)	Media (A)= 0.057, NAA(B)= 0.065, A×B=0.113			

Table 9: Effect of media type and NAA level (mgl^{-1}) on number of roots per explant.

Data in (Table 10) shows that average length of roots in Kainth is significantly affected by rooting media and NAA levels. As far as media is concerned, M_3 resulted in longer roots (11.04 mm) irrespective of NAA level. NAA at 0.1 mgl^{-1} resulted in significantly longer roots (20.53 mm) when compared to 16.97 obtained at NAA (1.0 mgl^{-1}).

Growth regulator (mgl^{-1})	Media			Mean
	M_1(1/2 MS)	M_2(MS)	M_3(WPM)	
NAA (0.1)	18.23	20.40	22.97	20.53
NAA (1.0)	14.33	15.37	21.20	16.97
NAA (2.0)	0.00	0.00	0.00	0.00
Control	0.00	0.00	0.00	0.00
Mean	8.14	8.94	11.04	
C.D($p \leq 0.05$)	Media (A)= 0.919, NAA(B)= 1.061, A×B=1.837			

Table 10: Effect of media type and NAA level (mgl^{-1}) on average length of roots (mm).

A treatment combination of M_3 fortified with NAA (0.1 mgl^{-1}) resulted in maximum average length of roots (22.97 mm) which is followed by 21.20 mm in M_3 medium fortified with NAA (1.0 mgl^{-1}). These two treatments were statistically at par in terms of controlling average root length.

Thakur [29] and Thakur and Kanwar [3] also reported better rooting response of Kainth as compared to scion varieties. Like the multiplication rate, rooting ability being genotype dependent [30] and rootstocks usually root with greater ability than scions [31]. The mineral concentration in the culture medium affects rooting characteristic and some researchers have proposed that reduction of salt strength to half strength improved rooting [32].

The reason behind increasing rooting rate on half strength culture medium might be due to a disorder in carbohydrate to nitrogen in nutrient medium, which lead to decreasing nitrogen level in shoot and then improving rooting rate, initiation roots, increasing root number and lengths [33]. Higher rooting response in Kainth using NAA than IBA is in conformity with [3,29]. Better rooting response of pear genotypes with NAA is in concordance to the findings of Singha [34] who preferred NAA over IBA for inducing roots in P. communis cv. Seckel to avoid the basal callus formation. Reed [35] 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 leads reduction in rooting by inducing basal callus formation [36] or by inhibiting the root elongation [37]. This may be the reason for poor rooting response at higher auxin concentration in the present study.

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