



MICROPROPAGATION OF KAINTH (*Pyrus pashia*) - AN IMPORTANT ROOTSTOCK OF PEAR IN NORTHERN SUBTROPICAL REGION OF INDIA

Rehman H U*, Gill M I S**, Sidhu G S and Dhaliwal H S

Department of Fruit Science, PAU, Ludhiana-141 004, India.

Received – April 07, 2014; Revision – April 18, 2014, Accepted – April 27, 2014
Available Online - April 30, 2014

KEYWORDS

Micropropagation

Nodal explants

Establishment

Proliferation

Necrotic culture

ABSTRACT

Micropropagation of Kainth using nodal explants 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. Per cent necrotic culture was found to be influenced by type of media and growth regulator fortification during establishment stage. Least necrotic culture percentage in Kainth was observed by using M_2 medium supplemented with BAP (1.5 mg l^{-1}) and IBA (0.01 mg l^{-1}). M_2 medium containing BAP (1.5 mg l^{-1}) and IBA (0.25 mg l^{-1}) gave maximum explant establishment (52.80 %). During proliferation stage, M_3 medium supplemented with BAP (3.0 mg l^{-1}) resulted in highest proliferated cultures (83.19%). Similarly the highest shoots per explant were obtained using M_3 medium supplemented with BAP (3.0 mg l^{-1}). However, shoots of maximum length were obtained in M_3 medium containing BAP (0.0 mg l^{-1}) i.e control. Per cent rooting, roots per explant and root length was found to be influenced by type of medium and growth regulator fortification. IBA (0.1 mg l^{-1}) induced maximum rooting (14.08 %) using M_1 medium however, NAA (1.0 mg l^{-1}) induced rooting of 27.46 per cent using M_1 medium. Similarly using IBA, maximum roots per explant were obtained using M_1 medium supplemented with IBA (1.0 mg l^{-1}). However, NAA (1.0 mg l^{-1}) induced highest roots per explant i.e 3.60 using M_2 medium. M_3 supplemented with IBA (0.1 mg l^{-1}) resulted in maximum root length of 31.10 mm however, NAA (1.0 mg l^{-1}) resulted in maximum root length of 23.22 mm using M_3 medium.

* Corresponding author

E-mail: haseebpom@gmail.com (Rehman H U) and misgill@pau.edu (Gill M I S)

Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

1 Introduction

Presently pear (*Pyrus spp*s) is next to apple in importance, acreage, production and varietal wealth among temperate fruits in India. It can grow under wider temperature conditions ranging from minus 26°C when dormant to as high as 45°C during growth period (Chadha, 2001). Pear is grown from warm humid sub-tropical plains to cold dry temperate regions of India occupying an area of 49340 ha with the annual production of 317270 MT (indiastat, 2013). The area can be increased further and cultivation of this crop may prove to be a best alternative for diversification of agriculture. Seedling rootstocks are not uniform in growth and productivity (Baviera et al., 1989). Therefore, vegetative propagation methods like cutting and stooling are used to multiply pear rootstocks. *In vitro* propagation 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 like *P betulaefolia* L. (Hassanen & Gabr 2012), wild pear (Thakur & Kanwar 2008), OPR 157, OPR 260 and OH × F 230 (Yeo & Reed 1995), *P calleryana* (Antunes de et al., 2004), Pyrodwarf (Ruzic et al., 2011) and *P communis* rootstock (Rahman et al., 2007).

2 Materials and Methods

Kainth plants growing in Fruit Research Farm, Department of Fruit Science, PAU, Ludhiana were used as a source of explants in the present study. Nodal segment explants were taken from current season's growth from March to November. The explant source was subjected to uniform cultural practices. The basal media used in the study were Murashige & Skoog's medium with half strength of macro and micronutrients (M_1), Murashige & Skoog's medium (M_2) and Woody Plant Medium (M_3).

Explants were first washed in running tap water for 15 minutes followed by keeping in 1 per cent bavistin along with few drops of Tween-20 for 20 minutes. Later on explants were washed thoroughly by keeping under running tap water till all residues gets washed out. Before culturing, explants were sterilized with 0.1 per cent $HgCl_2$ for 5 minutes within laminar air flow cabinet, followed by 3-4 washing using sterile water.

The hood of the cabinet was thoroughly cleaned by cotton dipped in methanol. All the instruments used were autoclaved before culturing. The explants were inoculated in test tubes/glass jars containing autoclaved media using sterilized forcep for establishment. Different media ($\frac{1}{2}$ MS, MS and WPM) fortified with different combinations of 6-benzylaminopurine (BAP) (0.5-3.0 $mg\ l^{-1}$) and IBA (0.01-2.0 $mg\ l^{-1}$) were used during establishment stage. The data were recorded on per cent necrotic cultures and explant establishment after 3 weeks of inoculation.

The explants which got established on medium were used as material for proliferation. Necessary dissection of established

explants was carried out using sterilized blade and forcep before transfer to shoot proliferation media. Various shoot proliferation media i.e. M_1 , M_2 and M_3 fortified with various concentrations of BAP i.e. 0.5-5.0 $mg\ l^{-1}$ were used. The optimum media and concentration of BAP for shoot proliferation was standardized. Observations on per cent proliferated cultures, number of shoots/explant and average length of shoot (mm) was recorded after third subculture and the culture duration were five weeks each.

After allowing shoots to multiply on shoot proliferation medium, individual shoots were separated ($>30mm$) and transferred to root regeneration medium. Different types of media i.e. M_1 , M_2 and M_3 containing various combinations of IBA (0.1-2.0 $mg\ l^{-1}$) and NAA (0.1-2.0 $mg\ l^{-1}$) were used. Observations on per cent rooting, number of roots/ explant and average length of roots (mm) were recorded four weeks after culturing. The data generated in course of the present study was analyzed using completely randomized design (factorial), replicated 3 times.

3 Results and Discussion

3.1 Effect of various media supplemented with growth regulators on necrotic cultures

The effect of different media and growth regulator combination on necrotic culture induction in Kainth explants during establishment stage is presented in Table 1. These data reveal exists the significance of the influence of media type and growth regulator concentration on per cent necrosis in Kainth explants during tissue culture. M_2 medium proved best in terms of least necrotic culture induction of 11.32 per-cent, followed by M_1 and M_3 which resulted in 14.79 and 14.98 per cent of necrotic cultures respectively. M_1 and M_3 medium were statistically similar in terms of induction of necrosis. The least per cent necrosis (6.35) was observed in media supplemented with BAP (1.5 $mg\ l^{-1}$) and IBA (1.0 $mg\ l^{-1}$), it was followed by combination of BAP (3.0 $mg\ l^{-1}$) and IBA (0.01 $mg\ l^{-1}$) resulting in 9.11 per cent of necrotic cultures. Cumulative effect of media and growth regulator revealed that the lowest necrotic culture (2.10 %) was observed by using M_2 medium fortified with BAP (1.5 $mg\ l^{-1}$) and IBA (0.01 $mg\ l^{-1}$) followed by M_2 fortified with BAP (3.0 $mg\ l^{-1}$) and IBA (0.01 $mg\ l^{-1}$). Maximum necrotic culture percentage of 28.66 was observed in M_1 medium fortified with BAP (0.5 $mg\ l^{-1}$) and IBA (2.0 $mg\ l^{-1}$). Almost all treatment combinations differ significantly in terms of induction of necrosis during establishment stage of *in vitro* culture. Although there is considerable work done on the relationship between necrosis and type and concentration of plant growth regulators, there is no definitive explanation for their *in vitro* effects (Bairu et al., 2009). But a variation observed in necrosis at various combinations of growth regulators has been credited to medium composition and growing conditions by Bairu et al. (2009).

Table 1 Effect of various media types and growth regulators (mg l^{-1}) on per-cent necrosis.

Growth regulator combination (mg l^{-1})	Media			Mean
	M ₁ (1/2 MS)	M ₂ (MS)	M ₃ (WPM)	
BAP(0.5)+IBA(0.01)	15.48	7.79	8.69	10.65
BAP(0.5)+IBA(1.0)	25.23	22.17	10.35	19.25
BAP(0.5)+IBA(2.0)	28.66	27.52	10.80	22.33
BAP(1.5)+IBA(0.01)	4.71	2.10	12.25	6.35
BAP(1.5)+IBA(1.0)	17.25	7.11	23.17	15.84
BAP(1.5)+IBA(2.0)	15.16	9.48	15.47	13.37
BAP(3.0)+IBA(0.01)	6.62	3.50	17.22	9.11
BAP(3.0)+IBA(1.0)	10.25	4.27	20.16	11.56
BAP(3.0)+IBA(2.0)	8.05	14.33	17.52	13.30
Control	16.45	14.89	14.19	15.18
Mean	14.79	11.32	14.98	
C.D ($p \leq 0.05$)	Media (A)=0.342, GR's (B)=0.706, A×B=1.296			

Equilibrium between phenolic compounds and hormones quite affects the success on plant tissue culture (Poessel et al., 1980). These results are in conformity with earlier observations made by De Paoli (1989) and Leite et al., (1997), who reported Murashige and Skoog medium as the best medium for culture initiation resulting in the highest per cent establishment with least necrotic cultures. Higher necrosis in Woody Plant Medium may be attributed due to lower total nitrogen content (Mamaghani et al., 2010).

3.2 Effect of medium on per cent explant establishment of Kainth

Highest establishment was reported in M₂ medium as shown in table 2. Explant establishment of 32.14 per-cent was achieved in M₂ medium and it was statistically higher as compared to M₁ and M₃ medium. Similarly, higher explant establishment (52.53 %) was reported from the media supplemented by BAP (1.5 mg l^{-1}) and IBA (0.25 mg l^{-1}). The interaction effect showed that the treatment combination of M₂ containing BAP (1.5 mg l^{-1}) and IBA (0.25 mg l^{-1}) gave maximum per-cent of explant

establishment (63.60) {Fig 1} whereas; minimum explant establishment of 10.68 per cent was obtained on M₃ supplemented with BAP (0.0 mg l^{-1}) and IBA (0.0 mg l^{-1}) i.e. control. 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 & Jiang (1993) in apple; Mondal et al., (1994) in *Carica papaya*; Caboni et al., (1999) in pear; Chakravarty & Goswami (1999) in *Citrus acida*; Akbar et al., (2003) in pineapple and Canli & Tian (2008) 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., (2013) reported better results in terms of establishment percentage in sweet cherry cv. Bigarreau Noir Grosse using Murashige & Skoog medium over Driver & Kuniyuli medium, Woody Plant Medium & Knop's macro and MS micro-organics medium independent of growth regulators concentration. *In vitro* effects of growth regulator on overall establishment of explant have been reported to be influenced by growth medium composition, growing conditions and genotype (Bairu et al., 2009, Karimpour et al., 2013).

Table 2 Effect of various media types and growth regulators (mg/l) on per cent explant establishment.

Growth regulator combination (mg l^{-1})	Media			Mean
	M ₁ (1/2 MS)	M ₂ (MS)	M ₃ (WPM)	
BAP(0.5)+IBA(0.01)	36.80	23.08	18.25	26.04
BAP(0.5)+IBA(0.25)	28.63	25.11	20.02	24.59
BAP(0.5)+IBA(0.5)	12.54	21.88	18.21	17.54
BAP(1.0)+IBA(0.01)	30.86	36.40	36.34	34.53
BAP(1.0)+IBA(0.25)	40.01	36.37	23.07	33.15
BAP(1.0)+IBA(0.5)	19.98	30.75	38.45	29.73
BAP(1.5)+IBA(0.01)	37.58	50.42	18.60	35.53
BAP(1.5)+IBA(0.25)	46.62	63.60	47.38	52.53
BAP(1.5)+IBA(0.5)	15.77	14.31	18.68	16.25
Control	13.40	19.45	10.68	14.51
Mean	28.22	32.14	24.97	
C.D ($p \leq 0.05$)	Media (A)=1.001, GR's (B)= 1.827, A×B=3.165			

3.3 *In vitro* shoot proliferation

Data regarding shoot proliferation which comprised of per cent proliferated culture, number of shoots per explant and average length of shoots was obtained after two sub culturing as shoot growth was very slow initially irrespective of media used and growth regulator level. Data of *in vitro* shoot proliferation has been given in Table 3, and these results clearly depicts that in Kainth, percentage of proliferated cultures was significantly affected by various treatment combinations. Statistically maximum proliferated cultures (59.09 %) were obtained on M₃ medium. With respect to BAP concentration, maximum proliferated cultures (64.79 %) were obtained by using BAP (3.0 mg l⁻¹), irrespective of media used. Similarly interaction values show that maximum proliferated cultures (83.19 %) were obtained by employing M₃ medium supplemented with BAP (3.0 mg l⁻¹) {Figure 2}.

From the perusal of data in Table 4, it is evident that number of shoots per explant is influenced by both treatments i.e. type of media and growth regulator concentration. The highest number of shoots per explant (4.10) was obtained when M₃ was used as shoot proliferation medium. M₁ and M₂ resulted in significantly lesser number of shoots per explants. Data in Table 4 clearly reveals that maximum number of shoots was obtained by using higher dose of BAP. Increasing BAP level from 0.5 mg l⁻¹ to 3.0 mg l⁻¹ resulted in increase in number of shoots per explant from 2.95 to 4.65. There existed a significant interaction between type of media and various levels of BAP by effecting number of shoots produced per explant. Maximum number of shoots per explant (5.93) was obtained in M₃ medium fortified with BAP (3.0 mg l⁻¹) followed by M₃ supplemented with BAP (1.5 mg l⁻¹).

Data regarding shoot length as influenced in by type of media and various doses of BAP is presented in Table 5. It is clearly evident that shoot length decreased with an increase in BAP dosage although number of shoots per explant increased significantly. Shoots of most desirable length were obtained on M₃ medium, which was significantly higher than on M₂ and M₁ medium. Data in Table 5 reveal that BAP (0.5 mg l⁻¹) is best in terms of production of the most desirable shoot length (41.47 mm), which is at par with control (39.97) containing no BAP. There was a significant interaction between type of media and BAP concentration for average length of shoots produced. Longest shoots (46.32 mm) were obtained in M₃ medium that was free of BAP followed by M₃ fortified with BAP (0.5 mg l⁻¹). These two treatments were significantly at par with respect to each other but were significantly better in terms of producing most desirable shoot length when compared with other treatment combinations. These findings are in conformity with those of Dwivedi & Bist (1999) in *P. pyrifolia* cv. Gola and Kadota et al. (2001) in Japanese pear cultivar Hosui, who reported superiority of WPM over other media with respect to proliferation rate. 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 Dwivedi & Bist (1999), Sedlak & Paprstein (2003), Cosac & Frasin (2008), Karimpour et al. (2013), Hassanen & Gabr (2012), Ruzic et al. (2011), Isikalan et al. (2011) and Soni et al. (2011). Hu & Wang (1983) 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 (2012) in Le Conte pear, Tange et al. (2008) in Bartlett pear and Mustafa et al. (2013) on fig due to higher nutrient concentration.

Table 3 Effect of media types and growth regulator level (mg l⁻¹) on per cent proliferated cultures.

Growth regulator (mg l ⁻¹)	Media			Mean
	M ₁ (1/2 MS)	M ₂ (MS)	M ₃ (WPM)	
BAP(0.5)	63.25	36.63	50.49	50.12
BAP(1.5)	45.12	52.92	73.32	57.12
BAP(3.0)	70.79	40.38	83.19	64.79
Control	11.73	14.35	29.36	18.48
Mean	47.72	36.07	59.09	
C.D (p≤0.05)	Media (A)= 2.352 , BAP (B)= 2.716, A×B=4.705			

Table 4 Effect of various media types and growth regulator level (mg l⁻¹) on number of shoots/explant.

Growth regulator (mg l ⁻¹)	Media			Mean
	M ₁ (1/2 MS)	M ₂ (MS)	M ₃ (WPM)	
BAP(0.5)	2.33	3.70	2.81	2.95
BAP(1.5)	2.90	4.23	5.13	4.09
BAP(3.0)	3.28	4.74	5.93	4.65
Control	1.54	2.23	2.51	2.09
Mean	2.51	3.73	4.10	
C.D (p≤0.05)	Media (A)= 0.126, BAP (B)= 0.145 , A×B=0.251			

Table 5 Effect of various media types and growth regulator level (mg l^{-1}) on average length of shoots (mm).

Growth regulator (mg l^{-1})	Media			Mean
	M ₁ (1/2 MS)	M ₂ (MS)	M ₃ (WPM)	
BAP(0.5)	40.65	40.35	43.40	41.47
BAP(1.5)	37.77	35.18	41.00	37.98
BAP(3.0)	28.25	32.22	36.97	32.48
Control	37.23	36.35	46.32	39.97
Mean	35.98	36.03	41.92	
C.D ($p \leq 0.05$)	Media (A)= 1.582, BAP (B)= 1.826 , A×B=3.163			

Table 6 Effect of various media types and IBA level (mg l^{-1}) on per cent rooting.

Growth regulator (mg l^{-1})	Media			Mean
	M ₁ (1/2 MS)	M ₂ (MS)	M ₃ (WPM)	
IBA (0.1)	14.08	8.90	8.39	10.46
IBA (1.0)	8.67	0.00	0.00	2.89
IBA (2.0)	0.00	0.00	0.00	0.00
Control	0.00	0.00	0.00	0.00
Mean	5.69	2.23	2.10	
C.D($p \leq 0.05$)	Media (A)= 0.814, IBA(B)=0.940, A×B=1.628			

Table 7 Effect of various media types and IBA level (mg l^{-1}) on number of roots per explant.

Growth regulator (mg l^{-1})	Media			Mean
	M ₁ (1/2 MS)	M ₂ (MS)	M ₃ (WPM)	
IBA (0.1)	1.98	1.86	1.65	1.83
IBA (1.0)	2.60	0.00	0.00	0.87
IBA (2.0)	0.00	0.00	0.00	0.00
Control	0.00	0.00	0.00	0.00
Mean	1.15	0.47	0.41	
C.D($p \leq 0.05$)	Media (A)= 0.053, IBA(B)= 0.061, A×B=0.106			

Table 8 Effect of various media types and IBA level (mg l^{-1}) on average length of roots (mm).

Growth regulator (mg l^{-1})	Media			Mean
	M ₁ (1/2 MS)	M ₂ (MS)	M ₃ (WPM)	
IBA (0.1)	23.30	29.00	31.10	27.80
IBA (1.0)	19.60	0.00	0.00	6.53
IBA (2.0)	0.00	0.00	0.00	0.00
Control	0.00	0.00	0.00	0.00
Mean	10.73	7.25	7.78	
C.D($p \leq 0.05$)	Media (A) = 0.856, IBA (B) = 0.988. A×B=1.711			

3.4 *In vitro* rooting of Kainth.

The *in vitro* regenerated shoots during shoot proliferation were transferred to various rooting media supplemented with different levels of IBA and NAA. Data pertaining to the per cent rooting, roots per explant and average length of roots (mm) as affected by type of media and IBA levels is presented in Tables 6, 7 and 8. The *in vitro* shoots obtained during proliferation stage were transferred to various rooting media supplemented with different levels of IBA (mg l^{-1}). Data of Table 6 clearly reveal that per cent rooting was effected

significantly by type of media and level of IBA. The highest level of rooting (5.67 %) was noticed in M₁ medium followed by M₂ (2.23 %) and lowest one reported in M₃ medium (2.10 %). Media supplemented by IBA (0.1 mg l^{-1}) favour rooting and highest rooting percentage (10.46) was obtained by this combination, which was significantly higher than rooting (%) at higher levels of IBA i.e. 2.89 per cent at IBA (1.0 mg l^{-1}). The highest rooting (14.08 %) was observed in M₁ medium fortified with IBA (0.1 mg l^{-1}) followed by 8.90 per cent in M₂ supplemented with IBA (0.1 mg l^{-1}) {Figure 3}.



Figure 1 Establishment of Kainth using M_2 fortified with BAP (1.5mg l^{-1}) and IBA (0.25mg l^{-1})

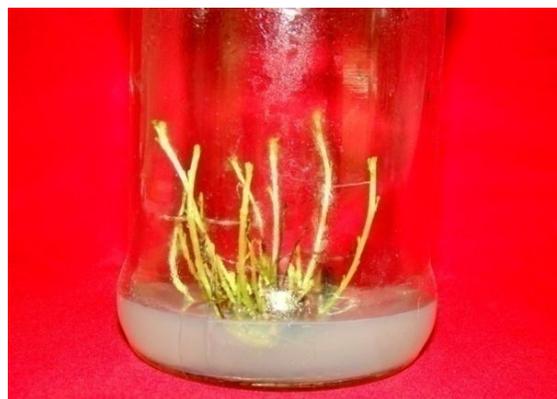


Figure 2 Proliferation of Kainth using M_3 fortified with BAP (3.0mg l^{-1})



Figure 3 Rooting in Kainth in M_1 containing IBA (0.1mg l^{-1})



Figure 4 Rooting in Kainth using M_1 fortified with NAA (1.0mg l^{-1})

Plate I Establishment, proliferation and rooting of Kainth.

Data in Table 7 represents the effect of rooting media and IBA levels (mg l^{-1}) on number of roots per explant. The highest number of roots per explant (1.15) was produced in M_1 , which is significantly higher than those obtained on M_2 and M_3 . Maximum number of roots per explant (1.83) was obtained by using IBA at 0.1 mg l^{-1} irrespective of media used. M_1 medium fortified with IBA (1.0 mg l^{-1}) resulted in significantly higher number of roots per explant (2.60) than rest of treatment combinations. Roots of maximum length (10.73 mm) were obtained in M_1 medium which is significantly higher than root length achieved in M_3 and M_2 medium (Table 8). Irrespective of media used, IBA (0.1 mg l^{-1}) resulted in longer roots (27.80 mm) than root length obtained at IBA (1.0 mg l^{-1}).

Interaction effect revealed that roots of maximum length (31.10 mm) were produced in M_3 medium fortified with IBA

(0.1 mg l^{-1}), which is significantly higher than other treatment combinations.

Tables 9, 10 and 11 shows the data regarding the effect of type of rooting media and various concentrations of NAA levels on rooting per cent, number of roots per explant and average length of roots (mm) respectively. Data in Table 9 clearly reveal per cent rooting induced by NAA was higher than rooting induced by IBA and rooting was effected significantly by type of media and level of NAA. The highest level of rooting (12.65 %) was noticed in M_1 medium followed by 11.83 per cent in M_2 and lowest (9.57 %) in M_3 medium. Rooting percentage of 22.90 was obtained using NAA (1.0mg l^{-1}) irrespective of type of rooting media, which is significantly at par to rooting obtained at NAA (0.1 mg l^{-1}) i.e. 22.51 %. The highest rooting (27.46 %) was observed in M_1 medium fortified with NAA (1.0 mg l^{-1}) {Figure 4} followed by 24.58

per cent in M₂ medium supplemented with NAA (1.0mg l⁻¹). Number of roots per explant was significantly higher by using M₂ medium irrespective of NAA fortification (Table 10). NAA at 1.0 mg l⁻¹ induced maximum number of roots per explant (2.84) followed by 2.63 using NAA (0.1 mg l⁻¹). An interaction between various media and NAA concentration on roots per explant was significant resulting in 3.60 roots per explant on M₂ medium supplemented with NAA (1.0 mg l⁻¹) followed by 3.26 roots per explant in M₁ fortified with NAA (1.0 mg l⁻¹). Similarly, root length was seen to be significantly affected by type of media and NAA level (Table 11). Statistically longer roots (8.32 mm) were observed on M₁ medium followed by M₂ and M₃, irrespective of NAA dosage. NAA at 0.1mg l⁻¹ resulted in longer average root length of 19.95 mm. Interaction values of average root length (mm) indicate that type of media and NAA levels has significant effect on this parameter. M₃ medium supplemented with NAA (1.0mg l⁻¹) resulted in longer roots (23.22 mm) followed by 21.56 mm in M₃ fortified with NAA (0.1mg l⁻¹). Thakur (2004) and Thakur & Kanwar (2008) also reported better rooting response of Kainth as compared to scion varieties. Like the multiplication rate, rooting ability being genotype dependent (Sharma et al. 2007) and rootstocks usually root with greater

ability than scions (Dobranszky & Teixeira da Silva, 2010). 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 (Dimassi-Theriou & Economou, 1993). 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 (Fotopoulos & Sotiropoulos 2005). Higher rooting response in Kainth by using NAA as compare IBA are in conformity with Thakur (2004) and Thakur & Kanwar (2008). Better rooting response of pear genotypes with NAA is in concordance to the findings of Singha (1980) who preferred NAA over IBA for inducing roots in *P. communis* cv. Seckel to avoid the basal callus formation. Reed (1995) 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 (Lane 1979) or by inhibiting the root elongation (Thimann 1977). This may be the reason for poor rooting response at higher auxin concentration in the present study.

Table 9 Effect of various media types and NAA level (mg l⁻¹) on per-cent rooting.

Growth regulator (mg l ⁻¹)	Media			Mean
	M ₁ (1/2 MS)	M ₂ (MS)	M ₃ (WPM)	
NAA (0.1)	23.15	22.74	21.63	22.51
NAA (1.0)	27.46	24.58	16.65	22.90
NAA (2.0)	0.00	0.00	0.00	0.00
Control	0.00	0.00	0.00	0.00
Mean	12.65	11.83	9.57	
C.D(p<0.05)	Media (A)=0.754, NAA(B)=0.871, A×B=1.508			

Table 10 Effect of various media types and NAA level (mg l⁻¹) on number of roots per explant.

Growth regulator (mg l ⁻¹)	Media			Mean
	M ₁ (1/2 MS)	M ₂ (MS)	M ₃ (WPM)	
NAA (0.1)	2.98	3.02	1.90	2.63
NAA (1.0)	3.26	3.60	1.66	2.84
NAA (2.0)	0.00	0.00	0.00	0.00
Control	0.00	0.00	0.00	0.00
Mean	1.56	1.66	0.89	
C.D(p<0.05)	Media (A)= 0.061, NAA(B)= 0.070, A×B=0.121			

Table 11 Effect of various media types and NAA level (mg l⁻¹) on average length of roots (mm).

Growth regulator (mg l ⁻¹)	Media			Mean
	M ₁ (1/2 MS)	M ₂ (MS)	M ₃ (WPM)	
NAA (0.1)	18.97	19.33	21.56	19.95
NAA (1.0)	14.30	14.22	23.22	17.25
NAA (2.0)	0.00	0.00	0.00	0.00
Control	0.00	0.00	0.00	0.00
Mean	8.32	8.39	11.20	
C.D(p<0.05)	Media (A)= 0.705, NAA(B)= 0.814, A×B=1.409			

References

- Akbar MA, Karmakar BK, Roy SK (2003) Callus induction and high frequency plant regeneration of pine apple (*Ananas comosus* L. Mers.) Plant Tissue Culture 13: 109-16.
- Antunes de MLK, Claudia F, Leandro C, Lima da SA (2004) *In vitro* establishment and multiplication of *Pyrus calleryana* D-6 on double-phase culture system. Brazilian Magazine of Fruit Culture 26: 403-05.
- Bairu MW, Jain N, Stirik WA, Dolezal K, Van Staden J (2009) Solving the problem of shoot-tip necrosis in *Harpagophytum procumbens* by changing the cytokinin types, calcium and boron concentrations in the medium. South African Journal of Botany 75: 122-27.
- Baviera JA, Garcia JL, Ibarra M (1989) Commercial *in vitro* micropropagation in pear cv. Conference. Acta Horticulturae 256: 63-68.
- Caboni E, Tonelli MG, Lauri P, Angeli SD, Damiano C (1999) *In vitro* shoot regeneration from leaves of wild pear. Plant Cell Tissue and Organ Culture 59: 1-7.
- Canli FA, Tian L (2008) *In vitro* shoot regeneration from stored mature cotyledons of sweet cherry (*Prunus avium* L.) cultivars. Scientia Horticulture 116: 34-40.
- Chadha TR (2001) Text book of Temperate Fruits. Indian Council of Agricultural Research, New Delhi.
- Chakravarty B, Goswami BC (1999) Plantlet regeneration from long term callus cultures of *Citrus acida* Roxb. and the uniformity of regenerated plants. Scientia Horticulture 82: 159-69.
- Cosac AC, Frasin LB (2008) *In vitro* propagation of some pear cultivars. Acta Horticulturae 800: 447-52.
- De Paoli G (1989) Micropropagation of pear cultivars. Informatore Agrario 43: 71-73.
- Dimassi-Theriou K, Economou A (1993). The effect of the explants type and the nutrient substrate composition on shoot production in *in vitro* cultures of the rootstock GF-677 (*P. persica* x *P. amygdalus*). In Scientific Annals. Aristotle University of Thessalonik pp. 31-35.
- Dobranszky J, Teixeira da Silva JA (2010). Micropropagation of apple- a review. Biotechnological Advances 28: 462-88.
- Dwivedi SK, Bist LD (1999) *In vitro* propagation of low-chill pear cv. Gola. Indian Journal of Horticulture 56: 189-93.
- Fan KH, Jiang ZT (1993) Studies on callus induction and plantlet regeneration from apple cotyledon. Journal of Shanghai Agricultural College 11: 243-48.
- Fotopoulos S, Sotiropoulos TE (2005) *In vitro* rooting of PR 204/84 rootstock (*Prunus persica* x *P. amygdalus*) as influenced by mineral concentration of the culture medium and exposure to darkness for a period Agronomy Research 3: 3-8.
- Hassan SAM (2012) Studies on improving flowering and fruiting of Leconte pears. Ph.D. Horticulture, Department, Faculty of Cairo University.
- Hassanen SA, Gabr MF (2012) *In vitro* propagation of pear (*Pyrus betulaeifolia*) rootstock. American-Eurasian Journal of Agriculture and Environmental Sciences 12 (4): 484-89.
- Hu CY, Wang P J (1983) Meristem, shoot tip and bud culture. In: D A Evans, W R Sharp, P V Ammirato and Y Yamada (eds.) Handbook of Plant cell culture vol I. MacMillan, New York, pp. 177-227.
- Indiastat (2013) Statewise area and production of various fruits in India. <http://indiastat.com>.
- Isikalan C, Namli S, Akbas F, Erol Ak B (2011) Micrografting of almond (*Amygdalus communis*) cultivar 'Nonpariel'. Australian Journal of Crop Science 5: 61-65.
- Kadota M, Imizu K, Hirano T (2001) Double-phase *in vitro* culture using sorbitol increases shoot proliferation and reduces hyperhydricity in Japanese pear. Scientia Horticulture 89: 207-15.
- Karimpour S, Davarynejad GH, Bagheri A, Tehranifar A (2013) *In vitro* establishment and clonal propagation of Sebrri pear cultivar. Journal of Agricultural Sciences and Technology. 15: 1209-17.
- Lane WD (1979) Regeneration of pear plants from shoot meristem tips. Plant Science Letters 16: 337-42.
- Leite GB, Finardi NL, Fortes GRL (1997) The effect of BAP and NAA concentration on multiplication "*in vitro*" of pears cv, Bartlett and clone OH x F 97. Ciencia-e-Agrotecnologia 21: 436-41.
- Mamaghani AB, Ghorbanli M, Assareh, MH, Ghamari ZA (2010) *In vitro* propagation of three Damask Roses accessions. Iranian Journal of Plant Physiology 1: 85-94.
- Mondal M, Gupta S, Mukhrejee BB (1994) Callus culture and plantlet production in *Carica papaya* (var. Honey Dew). Plant Cell Reproduction 18: 873-78.
- Mustafa NS, Rania A, Taha SAM, Hassan, Nagwa SMZ (2013) Effect of medium strength and carbon source on *in vitro* shoot multiplication of two *Ficus carica* cultivars. Journal of Applied Sciences Research 9: 3068-74.

- Peer FA, Rather ZA, Dar KR, Mir MA, Hussain G (2013) Studies of *in vitro* propagation of sweet cherry cv. Bigarreau Noir Grossa. *Indian Journal of Horticulture* 70: 317-22.
- Poessel JL, Martinez J, Mac Heix JJ, Jonard R (1980) Variations saisonnières de l' aptitude au greffage *in vitro* d' apex de pêcher (*Prunus persica* Batsch). Relations avec les teneurs en composés phénoliques et les activités peroxydasiques et polyphénoloxidasiques. *Physiologie Végétale* 18: 665-75.
- Rahman AE, Al-Ansary MF, Rizkalla AA, Badr-Elden AM (2007) Micropropagation and Biochemical Genetic Markers detection for Drought and Salt Tolerance of Pear Rootstock. *Australian Journal of Basic and Applied Sciences* 1: 625-36.
- Reed BM (1995) Screening *Pyrus* germplasms for *in vitro* rooting response. *Hort Science* 30: 1292-94.
- Ruzic DJ, Vujovic T, Nikolic D, Cerovic R (2011) *In vitro* growth responses of the 'Pyrodwarf' pear rootstock to cytokinin types. *Romanian Biotechnological Letters* 16: 6630-37.
- Sedlak J, Paprstein F (2003) Influence of growth regulators on *In Vitro* propagation of *Pyrus communis* cv. Koporecka. *Acta Horticulturae* 616: 25-29.
- Sharma T, Modgil M, Thakur M (2007). Factors affecting induction and development of *in vitro* rooting in apple rootstocks. *Indian Journal of Experimental Biology* 45: 824-29.
- Singha S (1980) *In vitro* propagation of 'Seckel' Pear. In : R H Zimmerman (ed.) Proceedings of the Conference on nursery production of fruit plants through tissue culture : applications and feasibility. USDA. pp. 59-63.
- Soni M, Thakur M, Modgil M (2011) *In vitro* multiplication of Merton I. 793-An apple rootstock suitable for replantation. *Indian Journal of Biotechnology* 10: 362-68.
- Tange H, Low Y, Lui C (2008) Plant regenerate from *in vitro* leaves of four commercial *pyrus* species. *Plant Soil Environments* 54: 140-48.
- Thakur A, Kanwar JS (2008) Micropropagation of 'wild pear' *Pyrus pyrifolia* (Burm F.) Nakai. II. Induction of rooting. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 36: 104-11.
- Thakur A (2004) Studies on *In vitro* clonal propagation and somatic cell culture in *Pyrus* species. Ph.D thesis submitted to Punjab Agricultural University, Ludhiana, Punjab.
- Thimann KV (1977) Hormone action in the life of plant. University of Massachusetts Press, Amherst. 510p.
- Yeo DY, Reed BM (1995) Micropropagation of three *Pyrus* rootstocks. *HortScience* 30: 620-23.