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In Vitro Culture Establishment of Patharnakh and Kainth As Effected By Explant Size and Media Type

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ABSTRACT: This study was carried in Tissue Culture Laboratory, Department of Fruit Science, Punjab Agricultural University, Ludhiana. The study revealed that explant size and medium had significant effect on per cent establishment in both Patharnakh and Kainth both individually as well as in interactive manner. Highest establishment in Patharnakh (78.63 %) and Kainth (69.96 %) resulted in Murashige and Skoog medium using 1.5-2.0 cm size explants.

KEYWORDS: Explant size, medium, establishment, Patharnakh, Kainth

I. INTRODUCTION

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 [1]. The chief pear growing areas are located in Jammu and Kashmir, Himachal Pradesh, Punjab, Uttarakhand, Arunachal Pradesh, Manipur, Mizoram, Nagaland and Tamil Nadu. In Punjab, it ranks 4th among fruit crops in terms of area after citrus, guava and mango and occupies an area of 2780 ha with an annual production of 62790 MT [1]. Current pear production relies on Patharnakh (hard pear) which is the leading cultivar of Punjab followed by Baggugosha and Leconte.

Tissue culture techniques are becoming increasingly popular means of plant vegetative propagation. In recent years, tissue culture technique e.g micro-propagation is increasingly used for rapid clonal multiplication of several economic plants, restoration of vigour and yield lost due to infection and preservation of germplasms. In a relatively short span of time and space, a large number of plants can be produced starting from a single individual.

II. LITERATURE SURVEY

Explant size and media has been reported to have significant influence on culture establishment in various woody plants especially fruit crops (14). Explants ranging from 0.2 mm to 20 mm have been used for micropropagation of pear. Zhao [2] cultured 0.5 mm shoot tips of *P. pyrifolia* cvs. Jinfeng and Zaosu for their *in vitro* propagation. Nicolodi and Pieber [3] used 0.2-0.3 mm long meristems with upto three leaf primodia for the micropropagation of *P. betulaefolia*. Banno et al. [4] used shoot tips (<0.5 mm) for micropropagation of six Asian pear cultivars. Shibli et al. [5] used shoot tips ranging from 0.5-0.7 mm in *P. syrica*. Bhojwani et al. [6] used 1.0 cm shoot tips in *P. pyrifolia*. Wang et al. [7] used 10-15 mm nodal segments for micropropagation of *P. bretschneideri*. Ten mm long nodal segments were used by Shen and Mullins [8] in *P. communis*. Similarly, 10-15 mm long shoot tips were used by Dwivedi and Bist [9, 10] for *in vitro* clonal multiplication of *P. pashia* and *P. pyrifolia* cv. Gola. Predieri and Govoni [11] used 15-20 mm shoot tips for the micropropagation of compact clones of *P. communis*. Twenty mm long shoot tips were used as explants for *in vitro* shoot proliferation of *P. communis* cv. Seckel [12].

Virtually all types of media has been tried for micropropagation of pear e.g Murashige and Skoog medium [13], revised Murashige and Skoog medium [14], Lepoivre medium [15], Driver- Kuniyuki Walnut medium and Woody Plant Medium [16], Cheng medium [17] etc.

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III. MATERIAL AND METHODS

Nodal segment explants of current season's growth of Patharnakh and Kainth were taken. Explants were first washed in running tap water 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. Various media like Murashige and Skoog's medium, Murashige and Skoog's medium with half strength of macro and micronutrients and Woody Plant Medium purchased in powdered form from Hi Media Private Limited. Prescribed amount of powder was dissolved in required amount of distilled water and fortified with $1.5mgl^{-1}$ 6-benzylaminopurine and $0.5mgl^{-1}$ Indolebutyric acid. Before autoclaving pH was adjusted to 5.8. Agar at the rate of $7.5gl^{-1}$ was dissolved by placing medium on gas burner. The medium was stirred regularly using glass rod to avoid agar clump formation till it started boiling. After allowing medium to cool for few minutes at room temperature, it was poured in culture tubes. The tubes were plugged using non-adsorbent cotton wrapped in muslin cloth. The medium was autoclaved at 15 psi and 121^{o}C. Media were allowed to solidify at room temperature. Before culturing, explants were cut in required size varying from 0.5- 3.0 cm and sterilized with 0.1 per cent $HgCl_2$ for 4 minutes within laminar air flow cabinet, followed by 3-4 washing using autoclaved distilled water. Observations on establishment (%) 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 using CPCS.

IV. RESULTS AND DISCUSSION

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

A Effect of explant size and media type on establishment of Patharnakh

From the perusal of the data in table 1, clearly reveals that explant size and media effects establishment (%) both individually and in interactive fashion. Maximum establishment (74.78 %) resulted by using 1.5-2.0 cm explants irrespective of media in Patharnakh. Significantly higher establishment (52.98 %) was obtained in Murashige and Skoog (MS) medium. Similar findings were reported by [14], [9], [10], [7], [11] and [12]. Establishment percentage was also found to be significantly affected in interactive manner by explant size and media type. Significantly maximum establishment resulted by using explant size of 1.5-2.0cm in MS media (Plate IA). Variation in per cent establishment of varied explant size may be due to different rate of callusing, contamination and variation in phenolic exudation [10, 14].

Table 1: Effect of explant size and media on establishment (%) in Patharnakh

Explant size (cm)	Media			Mean
	M_1 (1/2 MS)	M_2(MS)	M_3(WPM)	
0.5-1.0	30.84	36.28	31.10	**32.74**
1.0-1.5	41.26	52.36	47.53	**47.05**
1.5-2.0	70.89	78.63	74.81	**74.78**
2.0-2.5	61.42	56.42	57.13	**58.32**
2.5-3.0	39.93	41.19	38.06	**39.73**
Mean	**48.87**	**52.98**	**49.73**	
C.D($p \leq 0.05$)	**Explant size(A)= 1.38, Media (B)= 1.07, A×B= 2.40**			

B Effect of explant size and media type on establishment of Kainth

In Kainth, significantly the highest establishment (64.38 %) was observed by using explant size of 1.5-2.0cm. MS medium resulted in maximum establishment of 41.24 %, irrespective of explant size. Establishment (%) was also found to be significantly affected by explant size and media type in interactive manner. Highest establishment (69.96 %) was obtained by using explant size of 1.5-2.0 cm and MS medium (Plate IB).Variation in per cent establishment of Kainth as compared to Patharnakh may be due to different genotype, callusing, phenolic exudation and auxin-cytokinin ratio of explant used for culturing [14, 18].

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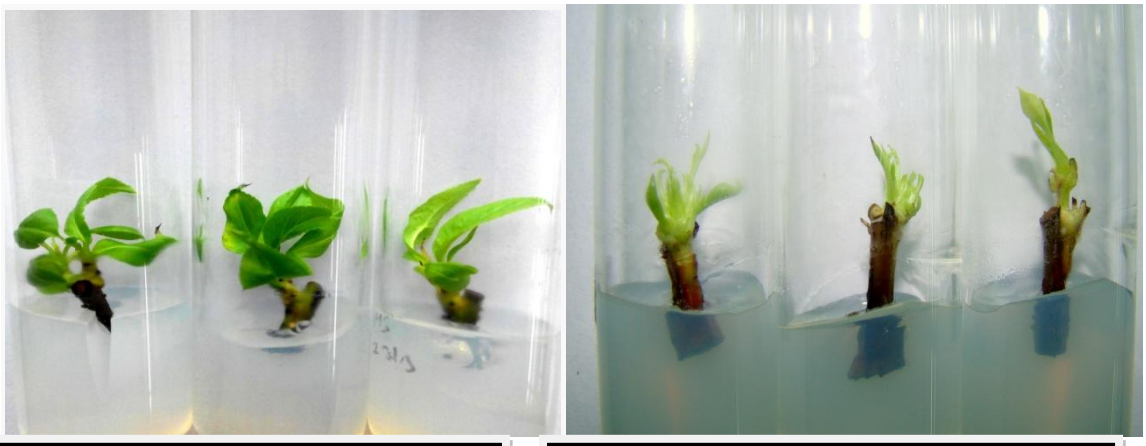
Table 2: Effect of explant size and media on establishment (%) in Kainth

Explant size (cm)	Media			Mean
	M_1 (1/2 MS)	M_2(MS)	M_3(WPM)	
0.5-1.0	20.84	24.42	21.86	**22.37**
1.0-1.5	31.60	44.77	39.18	**38.52**
1.5-2.0	64.84	69.96	58.34	**64.38**
2.0-2.5	57.16	37.92	35.69	**43.59**
2.5-3.0	21.74	29.11	11.41	**20.75**
Mean	**39.24**	**41.24**	**33.30**	
C.D(p≤0.05)	**Explant size(A)= 1.91, Media (B)= 1.48, A×B= 3.30**			



A: Establishment of 1.5-2.0 cm explants of Patharnakh on MS medium

B: Establishment of 1.5-2.0 cm explants of Kainth on MS medium

Plate I: Establishment of Patharnakh and Kainth

V. CONCLUSION

The study revealed that explant size and medium had significant effect on per cent establishment in both Patharnakh and Kainth both individually as well as in interactive manner. Highest establishment in Patharnakh (78.63 %) and Kainth (69.96 %) resulted in Murashige and Skoog medium using 1.5-2.0 cm size explants.

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