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Effect of surface sterilizing agents on in vitro culture establishment of Patharnakh *(Pyrus pyrifolia)* and Kainth *(Pyrus pashia)*

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Abstract

This experiment was done during 2011-13 in Tissue culture laboratory in Department of Fruit Science, PAU, Ludhiana. Nodal segments were obtained from shoots put forth by the sprouted buds of forced cuttings of Patharnakh and Kainth grown in growth chamber. Two sterilising agents namely mercuric chloride ($HgCl_2$) and sodium hypochlorite ($NaOCl_2$) at varied concentrations and durations were tested for sterilization of Patharnakh and Kainth. The results indicated that $HgCl_2$ proved better in terms of aseptic culture (%) and survival (%) than $NaOCl_2$ in both Patharnakh and Kainth. $HgCl_2$ (0.2%) proved better than $HgCl_2$ (0.1 %) resulting in higher aseptic culture in Patharnakh (66.56 %) and Kainth (51.36 %). Five minute treatment of $HgCl_2$ (0.2 %) resulted in significantly higher aseptic cultures in Patharnakh (89.49 %) and Kainth (94.85 %) than other durations. However, significantly higher explant survival of Patharnakh (78.71 %) and Kainth (72.30 %) was obtained by using $HgCl_2$ (0.1%) for 5 minutes.

Keywords: Nodal segments, Patharnakh, Kainth, aseptic cultures, explant survival.

Introduction

Among pear cultivars Patharnakh is the leading cultivar of Punjab contributing more than 80 per cent of pear production. Besides Patharnakh other pear cultivars grown under sub tropical conditions of Punjab includes Baggugosha, Leconte, Punjab Beauty, Punjab Nectar, Punjab Gold, YaLi, Nijjisseiki etc. Kainth is considered as the most important rootstock under Punjab conditions. Seedling rootstocks are not uniform in growth and productivity [1]. 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. Plant parts used for clonal propagation carry a wide range of contaminants and hence obtaining sterile plant material is very difficult [2]. Therefore, explants need surface sterilization before culturing besides, *in vitro* propagation provide suitable environment for growth of fungus and bacteria, unsuccessful sterilization hinders the progress of micropropagation studies. The kind, concentration and duration of disinfection treatment depend upon the degree of contamination and the hardiness of explant. Many sterilizing agents such as chlorine water, bleaching water, mercuric chloride, hydrogen peroxide, sodium hypochlorite etc have been used. This treatment also caused leaching of water soluble phenols and other growth inhibitors [3] and effectively reduced the infection [4].

Material and methods

For obtaining explants, the dormant cuttings from Patharnakh and Kainth plants were subjected to forcing treatment. For forcing dormant cutting (terminal and sub-terminal) 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 glass jars containing sterile distilled water, 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. The water in glass jars was changed every 4-5 days. Shoots put forth by the sprouted buds served as explant during culture establishment. A crop of explants became ready for first harvesting within 25 ± 5 days for Patharnakh and 20 ± 5 days for Kainth. After harvesting first crop of explants, the cuttings were again ready for harvesting of second crop within 10 ± 5 days.

Murashige and Skoog medium (MS) fortified with 6-benzylaminopurine ($1.5mgl^{-1}$) and Indolebutyric acid ($0.5mgl^{-1}$) was used for culture establishment. 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

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poured in culture tubes. These culture tubes were autoclaved at 15 psi and 121°C. Media were allowed to solidify at room temperature. 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 $HgCl_2$ (0.1, 0.2 %) for 1 to 5 minutes and $NaOCl_2$ (0.5, 1.0 %) for 2 to 10 minutes within laminar air flow cabinet, followed by 3-4 washing using autoclaved distilled water. Observations on aseptic culture (%) and survival (%) were recorded four weeks after culturing. The data generated in course of the present study was analyzed using completely randomized design (factorial) using CPCS software.

Results and discussion

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

Effect of sterilizing agents on *in vitro* culture establishment of Patharnakh

Table 1 shows the data regarding the effect of different sterliants namely $HgCl_2$ and $NaOCl_2$ and their concentration along with their time of exposure on per cent aseptic culture and per cent explant survival. Data clearly reveals that $HgCl_2$ treatment results in better results over $NaOCl_2$ in terms of higher aseptic culture (57.52 %) and survival (36.52 %), irrespective of concentration and time of exposure. Significantly higher aseptic cultures (66.56 %) and survival (38.15 %) resulted by using 0.2 per cent $HgCl_2$, irrespective of time of exposure. Highest aseptic cultures (89.49 %) resulted by using $HgCl_2$ (0.2 %) for 5 minutes, however maximum survival (78.71 %) was obtained by using $HgCl_2$ (0.1 %) for 5 minutes. Higher per cent aseptic cultures using $HgCl_2$ is in concordance with earlier studies carried by [5], [6], [7] and [8]. More survival of explants using lower concentrated $HgCl_2$ solution as compared to higher dose is in conformity with [5], [6] and [9]. However, $NaOCl_2$ has been reported to be better sterilising agent as compared to $HgCl_2$ in controlling the infection of potato cv. Kufri Himalini [10]. This difference reported might be due to different genotype, hardiness and explant used for *in vitro* propagation [5]. The genotypic variations with regard to per cent explant survival may be due to variation in phenol exudation resulting in browning of explants. However, both sterilizing agents, $HgCl_2$ and $NaOCl_2$, were effective in making clean explants of Kinnow tree [11]. Lower explant survival (%) using $HgCl_2$ (0.2 %) for longer duration may be due to phytoxicity by mercury present in mercuric chloride [6, 9].

Effect of sterilizing agents on *in vitro* culture establishment of Kainth

The data regarding the effect of sterilizing agents, their concentration and time of exposure on per cent aseptic culture and per cent survival during culture establishment in Kainth is presented in Table 2. As in Patharnakh, $HgCl_2$ results in higher aseptic culture (45.58 %) and survival (33.10 %) of Kainth explants, irrespective of its concentration and exposure time than $NaOCl_2$. $HgCl_2$ (0.2 %) proved better as compared to $HgCl_2$ (0.1 %) resulting in higher aseptic cultures (51.36 %) and survival (37.87 %), irrespective of time of exposure. With respect to $HgCl_2$ concentration and exposure time, $HgCl_2$ (0.2 %) for 5 minutes resulted in higher aseptic cultures (94.85 %). However, $HgCl_2$ (0.1 %) for 5 minutes resulted in significantly higher explant survival (72.30 %). Although Kainth followed similar to Patharnakh to sterliant treatment but variation observed in aseptic culture (%) and survival (%) may be due to different genotype and different level of hardiness affecting final results [2, 5].

Table 1: Effect of surface sterliants and duration of exposure on asepsis of culture and survival of nodal segment explants in Patharnakh

Surface sterliant (%)	Exposure time (min)	Aseptic culture (%)	Survival (%)
$Hgcl_2$(0.1)	1	27.78	9.97
	2	34.96	13.51
	3	44.99	27.71
	4	50.46	44.55
	5	84.23	78.71
	Mean (0.1$HgCl_2$)	**48.48**	**34.89**
$Hgcl_2$(0.2)	1	32.32	10.49
	2	49.80	23.56
	3	77.77	72.88
	4	83.41	55.56
	5	89.49	28.25

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	Mean (0.2$HgCl_2$)	**66.56**	**38.15**
Overall mean ($HgCl_2$)		**57.52**	**36.52**
$NaOCl_2$(0.5)	2	0.00	0.00
	4	0.00	0.00
	6	0.00	0.00
	8	17.34	14.92
	10	32.97	18.39
	Mean (0.5$NaOCl_2$)	**10.06**	**6.66**
$NaOCl_2$(1.0)	2	0.00	0.00
	4	0.00	0.00
	6	28.17	16.59
	8	39.38	33.96
	10	55.11	21.69
	Mean (1.0$NaOCl_2$)	**24.53**	**14.45**
Overall mean ($NaOCl_2$)		**17.30**	**10.56**
C.D(0.05)		Sterliant (A)= 1.05 Exposure time (B)= 1.66 A×B= 2.35	Sterliant (A)= 1.00 Exposure time (B)= 1.58 A×B= 2.23

Table 2: Effect of surface sterliants and duration of exposure on per cent aseptic culture and per cent survival of nodal segment explants in Kainth

Surface sterliant (%)	Exposure time (min)	Aseptic culture (%)	Survival (%)
$HgCl_2$(0.1)	1	0.00	0.00
	2	17.70	12.49
	3	40.37	22.85
	4	56.71	33.97
	5	84.20	72.30
	Mean (0.1$HgCl_2$)	**39.80**	**28.32**
$Hgcl_2$(0.2)	1	0.00	0.00
	2	27.61	11.11
	3	50.43	39.49
	4	83.90	77.38
	5	94.85	61.37
	Mean (0.2$HgCl_2$)	**51.36**	**37.87**
Overall mean ($HgCl_2$)		**45.58**	**33.10**
$NaOCl_2$(0.5)	2	0.00	0.00
	4	0.00	0.00
	6	0.00	0.00
	8	17.41	5.38
	10	22.89	6.63
	Mean (0.5$NaOCl_2$)	**8.06**	**2.40**
$NaOCl_2$(1.0)	2	0.00	0.00
	4	0.00	0.00
	6	66.46	56.79
	8	73.26	50.41
	10	74.11	37.44
	Mean (1.0$NaOCl_2$)	**42.77**	**28.93**
Overall mean ($NaOCl_2$)		**25.42**	**15.67**
C.D(0.05)		Sterliant (A)= 1.06 Exposure time (B)= 1.67 A×B= 2.36	Sterliant (A)= 1.16 Exposure time (B)= 1.84 A×B= 2.60

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Conclusion

The present study "Effect of surface sterilizing agents on *in vitro* culture establishment of Patharnakh (*Pyrus pyrifolia*) and Kainth (*Pyrus pashia*) was carried to standardize the type, concentration and time of exposure of sterliant so that least contamination and maximum survival can be achieved. The study concluded that $HgCl_2$ (0.2 %) used for 5 minute treatment was significantly better than rest of treatment combination in terms of per cent aseptic culture in both Patharnakh and Kainth. However, $HgCl_2$ (0.1 %) used for 5 minutes resulted significantly higher per cent survival in Patharnakh and Kainth.

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