International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(4): 1744-1747

Received: 12-05-2017
Accepted: 14-06-2017

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Effects of surface sterilization agents on *in vitro* plant growth in banana cultivar "Grand Naine"

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Abstract
In the present study, the effects of three different surface sterilization agents i.e. mercuric chloride, bavistin and ethanol were tested on the contamination-free establishment of banana cv. Grand Naine under *in vitro* conditions. All the sterilization agents performed better results when used individually for different time intervals. The combination of 0.1% $HgCl_2$ with 70% ethanol was also found effective for sterilization of banana explants. Best results with lower contamination and higher explant survival % were recorded with 0.1 % $HgCl_2$. The present study concludes the use of 0.1 % $HgCl_2$ for different time intervals to generate contamination-free plants in banana cv. Grand Naine with higher explant survival percentage.

Keywords: Aseptic Technique, Explant, *In-Vitro*, Musa, Sucker

Introduction
Banana is a member of family *Musaceae* that is originated in the hot tropical regions of South East Asia. India produces about 11% of world's banana. India accounts for 858.0 thousand hectares area under the cultivation of banana with an annual production of about 29163.0 thousand metric tonnes with an average productivity of 34.0 MT/ha (NHB, 2016) [14]. Banana is a rich source of carbohydrate, vitamin B, potassium, phosphorus, calcium and magnesium. It is easily digestible fruit and reduces the risks of several diseases including heart diseases, arthritis, ulcers, gastroenteritis and kidney disorders (Anonymous, 2013) [1]. The propagation of banana cultivars through tissue culture techniques has gained so much attention in recent years due to potential of these techniques to generate genetically identical and disease free quality planting materials. *In vitro* clonal propagation of banana using shoot tip cultures has been established earlierly (Cronauer and Krikorian, 1984; Banerjee, 1985) [4, 3]. Micropropagation has been a potential strategy for banana multiplication at commercial scale globally and it is estimated that each year more than 25 million plantlets are produced through this technology. Earlier studies show that micropropagated banana plants have the ability to establish faster, grow more vigorously and have higher yields than conventional suckers (Jarret *et al*., 1985) [7]. The basic step in micropropagation is the *in vitro* establishment of contamination-free plantlets. This could be easily achieved by using effective chemical sterilization procedures. The investigation of the effects of different surface sterilization agents on culture establishment and explant survival has been the prior requirement of banana micropropagation. Therefore, the present study was designed to conduct such type of experiments for developing efficient sterilization procedure for *in vitro* clonal propagation of banana cv. Grand Naine with lower contamination rates and higher explant survival percentages.

Material and Methods
The present study was carried out at the Tissue Culture Laboratory, Department of Horticulture, Sardar Vallabhbhai Patel University of Agriculture & Technology, Modipuram, Meerut, Uttar Pradesh for developing efficient sterilization procedure for the *in vitro* establishment of contamination-free plantlets of Banana cv. Grand Naine. The sword suckers from field grown Banana cv. Grand Naine were used as explants to investigate the effects of different surface sterilization agents such as Mercuric chloride, Bavistin (0.1%) and Ethanol

(70%). Firstly, the suckers were washed by soap under tap water and the outer layer was removed carefully. To remove soap and soil, explants were put under running tap water for 30 min and rinsed 3 times by distilled water. After that, the sucker explants were soaked in 100% Clorox and three drops of Tween-20 solution for 15 minutes under the hood of laminar flow. The explants were rinsed with sterilized distilled water for three times, followed by soaking in Mercuric chloride, Bavistin (0.1%) and Ethanol (70%) for different time intervals. As the final step, the suckers were rinsed by sterilized distilled water for three times, and were trimmed, cut and cultured in MS media. All needed glassware, equipment and distilled water were autoclaved at 121°C for 20 min. The inside surface of laminar flow was wiped by 70% ethanol and was sterilized by UV for 15 min prior to explant sterilization. Finally, all explants inoculated on basal MS media (Murashige and Skoog, 1962) [12] supplemented with 4.0 mg/L BAP and 1.5 mg/L Kinetin were incubated in culture room at 25ºC with 60% relative humidity at 16h/8h light/dark photoperiods under white fluorescent tubes providing light intensity of 4000 lux. The contamination percentage and explant survival were recorded at weekly time intervals and the contaminated cultures were discarded immediately. All the experiments were conducted in a complete randomized design (CRD) with a minimum of ten replicates (n=10) per treatment and repeated thrice. One replicate means one culture vessel. The effect of different treatments on various parameters was quantified and the significance of difference among means was determined by analysis of variance (ANOVA) using SPSS version 16.0 (SPSS, Chicago, USA) followed by Duncan's New Multiple Range Test (DMRT) at $p < 0.05$.

Results and Discussion

As the data presented in table-1, the maximum contamination percentage of explants after 15 days (76.00%) was noted under the treatment of $HgCl_2$ 0.1% for a period of 2 min. followed by 60.00, 45.66 and 33.33 percent with the duration of 4, 6 and 8 min, while the minimum (22.33%) was noted under $HgCl_2$ 0.1% treating for a period of 10 min. A critical observation was recorded as the maximum survival percentage of explants after 30 days (87.66%) was noted under the treatment of $HgCl_2$ 0.1% for a period of 2min.followed by 76.66, 55.66 and 40.00 percent with the duration of 4, 6 and 8 min. while the minimum (24.00%) was noted under $HgCl_2$ 0.1% treating for a period of 10 min. So, it was observed that survival percentage of explants was *vice versa* to the duration of the treatment with $HgCl_2$ (0.1%).

The data given in table-2 elucidate that the maximum contamination percentage among explants after 15 days (76.33%) was noted under the treatment of bavistin 0.1% for a period of 2 min followed by 60.00, 46.33 and 37.33 percent with the duration of 4, 6 and 8 min, while the minimum (26.66%) was noted under bavistin 0.1% treating for a period of 10 min. A critical observation was recorded as the maximum survival percentage of explants after 30 days (86.33%) was noted under the treatment of bavistin 0.1% for a period of 2 min. followed by 77.66, 57.33 and 40.33 percent with the duration of 4, 6 and 8 min. while the minimum (28.66%) was noted under bavistin 0.1% treating for a period of 10min. So, it was observed that survival percentage of explants was again *vice versa* to the duration of the treatment with bavistin (0.1%). It is clear from the data presented in table-3 that the maximum contamination percentage of explants after 15 days (79.33%) was noted under the treatment of Ethanol (70%) for a period of 2 min followed by 63.33, 49.66 and 40.00 percent with the duration of 3, 4 and 5min. while the minimum (30.00%) was noted under Ethanol (70%) treating for a period of 6 min. A critical observation was recorded as the maximum survival percentage of explants after 30 days (86.66%) was noted under the treatment of Ethanol (70%) for a period of 2 min. followed by 78.66, 58.66 and 44.66 percent with the duration of 3, 4 and 5 min. while the minimum (34.00) was noted under Ethanol (70%) treating for a period of 6min. So, it was observed that survival percentage of explants was again *vice versa* to the duration of the treatment with Ethanol (70 %). Data of table-4 showed the maximum contamination percentage of explants after 15 days (85.33%) under the treatment of Mercuric Chloride (0.1%) + Ethanol (70%) for a period of 1 min followed by 63.33, 42.66 and 42.33 percent with the duration of 2, 3 and 4 min. while the minimum (15.66%) was noted under Mercuric Chloride (0.1%) + Ethanol (70%) treating for a period of 5 min. A critical observation was recorded as the maximum death percentage of explants after 20 days (24.66%) was noted under the treatment of Mercuric Chloride (0.1%) + Ethanol (70%) for a period of 5 min. followed by 24.33, 23.33 and 11.00 percent with the duration of 5, 4 and 3 min. while the minimum (10.00%) was noted under Mercuric Chloride (0.1%) + Ethanol (70%) treating for a period of 1min. So, it was observed that death percentage of explants was successive to the duration of the treatment with Mercuric Chloride (0.1 %) + Ethanol (70%).Further, a minute observation was recorded as the maximum survival percentage of explants after 30 days (74.33%) was noted under the treatment of Mercuric Chloride (0.1%) + Ethanol (70%) for a period of 1 min. followed by 34.00, 33.00 and 12.33 percent with the duration of 2, 3 and 4 min. while the minimum (3.66%) was noted under Mercuric Chloride (0.1%) + Ethanol (70%) treating for a period of 5 min. So, it was observed that survival percentage of explants was *vice versa* to the duration of the treatment with Mercuric Chloride (0.1%) + Ethanol (70%). Amongst the three sterilants tested in the present study, Ethanol (70%) was found better for controlling the infection and it had not any adverse effect on explants even in long duration (1-2 minutes) exposure. However, a treatment combination of Mercuric Chloride (1.0%) with Ethanol (70%) for 1 minute followed by $HgCl_2$ (0.1%) + Ethanol (70%) for 2 minutes resulted the highest percentage (74% and 34%) of aseptic culture establishment in banana cv. Grand Naine. The same pattern was observed to reduce microorganism and sterilize the explant to get a clean material for *in-vitro* propagation of banana by Mendes *et al.*, 1996 [9], Baneqee and Sharma, 1988 [2], Silva *et al.*, 1998 [17], Nandwani *et al.*, 2000 [13], Rahman *et al.*, 2002 [16], Habiba *et al.*, 2002 [5], Madhulatha *et al.*, 2004 [8], Molla *et al.*, 2004 [10], Muhammad *et al.*, 2004 [11], Titov *et al.*, 2006 [18]. Ethanol with low concentration of $HgCl_2$ has also been used by a number of research workers for disinfection purposes (Silva *et al.*, 1998, Rahman *et al.*, 2002, Jalil *et al*, 2003, Onuoha *et al.*, 2011) [17, 16, 6, 15].

Table 1: Standardization of $HgCl_2$ (0.1%) treatment duration for surface sterilization of shoot tips of Grand Naine

Treatments	Percentage of explants contaminated after 15 days		Percentage of explants survived after 30 days	
2 min	76.000	a	87.667	a
4 min	60.000	b	76.667	b
6 min	45.667	c	55.667	c
8 min	33.333	d	40.000	d
10 min	22.333	e	24.000	e
Gen. Mean	47.467	***	56.800	***
C.V.	6.595		6.149	
S.E.M.	1.807		2.017	
C.D. @ 5%	5.695		6.354	
C.D. @ 1%	8.101		9.038	

Table 2: Standardization of Bavistin (0.1%) treatment period for surface sterilization of shoot tips of banana cv. Grand Naine

Treatments	Percentage of explants contaminated after 15 days		Percentage of explants survived after 30 days	
2 min	76.333	a	86.333	a
4 min	60.000	b	77.667	b
6 min	46.333	c	57.333	c
8 min	37.333	d	40.333	d
10 min	26.667	e	28.667	e
Gen. Mean	49.333	***	58.067	***
C.V.	7.041		6.610	
S.E.M.	2.006		2.216	
C.D. @ 5%	6.320		6.983	
C.D. @ 1%	8.989		9.933	

Table 3: Standardization of Ethanol (70%) treatment period for surface sterilization of shoot tips of banana cv. Grand Naine.

Treatments	Percentage of explants contaminated after 15 days		Percentage of explants survived after 30 days	
2 min	79.333	**a**	86.667	**a**
3 min	63.333	**b**	78.667	**b**
4 min	49.667	**c**	58.667	**c**
5 min	40.000	**d**	44.667	**d**
6 min	30.000	**e**	34.000	**e**
Gen. Mean	**52.467**	*******	**60.533**	*******
C.V.	6.657		5.643	
S.E.M.	2.017		1.972	
C.D. 5%	6.354		6.214	
C.D. 1%	9.038		8.839	

Table 4: Standardization of surface sterilization treatment for banana explants cv. Grand Naine

Mercuric Chloride (0.1 %) + Ethanol (70 %)	Contamination % at 15 days		Death of culture % at 20 days		Explants survival % at 30 days	
1 min	85.333	a	10.000	b	74.333	a
2 min	63.333	b	11.000	b	34.000	b
3 min	42.667	c	23.333	a	33.000	b
4 min	42.333	c	24.333	a	12.333	c
5 min	15.667	d	24.667	a	3.667	d
Gen. Mean	49.867	***	18.667	***	31.467	***
C.V.	4.966		8.299		6.666	
S.E.M.	1.430		0.894		1.211	
C.D. @ 5%	4.506		2.818		3.816	
C.D. @ 1%	6.409		4.009		5.428	

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