

Original Research Article

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## Assessment of Various Sterilants on Differential Growth Response in *Carica papaya*

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### ABSTRACT

Papaya is a nutraceutical fruit crop grown among tropical and sub-tropical regions of the world. The propagation through seeds results in the production of non-true-type planting materials due to segregation of off-springs at second filial generation leading to inherent heterozygosity. The alternative strategy to this is in-vitro propagation, but the main hurdles comes under this method is failure to culture establishment. Thus, searching for an effective sterilization is always needed. Therefore, present study was intended to evaluate the effect of various sterilant on culture establishment. The lateral buds were collected as explant and treated with 0.1% Bavistin, 0.1% HgCl<sub>2</sub>, 4% NaOCl and 70% EtOH for different time interval. The higher survival percent and uniform growth pattern was observed with 0.1% Bavistin for 10 min (38.7), 0.1% HgCl<sub>2</sub> for 2 min (40.5), 4% NaOCl (39) and 70% EtOH for 1 min (37.8), whereas combination of these sterilant showed 46.3% shoot survival with uniform growth.

#### Keywords

Sterilant,  
Contamination,  
Papaya, Survival

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### Introduction

Papaya is a nutraceutical important fruit crop grown over tropical and sub-tropical region of

the world (Jaime *et al.*, 2007; Ryavalad *et al.*, 2019). In India, papaya represented by four species in which *Carica papaya* L. is the most widely cultivated and the best known species

(Jean *et al.*, 2011; Vij and Prashar, 2015). This crop is cultivated over 71 countries of the world, and India apart its major part in respect to the area (13400 ha), yield (443284 hg/ha) and production (5940000 tonnes) (FAOSTAT, 2017). At conventional scale, it is propagated through seeds (Fernando *et al.*, 2001), but the set back of seed propagation is the production of nontrue-to-type planting materials (Panjaitan *et al.*, 2007; Tsai *et al.*, 2009) due to segregation of off-springs at second filial generation that leads to inherent heterozygosity (Veerannale, 1984; Rajeevan and Pandey, 1986) and high percentages of unwanted male plants (Farzana *et al.*, 2008). Papaya plants can also be grown via asexual means (cutting and grafting) but these methods are very tedious and impractical when carried out at large scale (Saker *et al.*, 1999). Moreover, these methods cannot be used to prevent transmission of systemic disease from mother plants (Van-Hong *et al.*, 2018). Now days, tissue culture techniques had witnessed as an efficient technique for the multiplication of specified sex type plants, production of true-to-type, disease-free plantlets and in the genetic transformation studies. The foremost obstacle emanates to *in-vitro* propagation is failure to culture establishment due to microbial contamination by surface and endogenous microbes when explants collected from mature field grown plants (Wu *et al.*, 2012; Kumar *et al.*, 2019).

The collected explants from field conditions comprehends huge number of microbes on their surface. During *in-vitro* propagation, fiasco in ample exclusion of these contaminants affects explant/ shoot sustenance and strive with inoculated explant for sustenance (Kumar *et al.*, 2019). Contagious contaminations diminish the impermanence of *in-vitro* cultures and cause necrosis. The explant inoculated on medium skipping any sterilant treatment consequences in a reduced amount of culture sustenance, sometimes no culture survival. The

pervasiveness of uncleanness makes culture establishment vulnerable in accomplishing goal line. The effectiveness of *in-vitro* culture establishment needs preliminary consideration to explant assortment following an effective treatment of sterilizing agents.

The assortment of sterilizing agents should be the kind which could eliminate wide range of contamination (Panhwar, 2005), and confirm higher culture subsistence. The successfulness of sterilization protocol is the key point to circumvent the incursion and growth of contaminants. Thus, better optimization of sterilant combination is always needed to accomplish sustainable micropropagation. Therefore, study was intended to assess the various sterilant and their combination on explant survival and shoot growth using lateral bud as explant.

## **Materials and Methods**

The current study was conducted at Tissue culture laboratory, Department of Agricultural Biotechnology, College of Agriculture, SVPUA&T, Meerut. The explant for current study were collected from field grown mature papaya plants in the form of lateral buds (Fig. 1). The propagation medium was prepared by augmenting with 30 g/L sucrose, 100mg/L polyvinyl pyrrolidone, 100 mg/L myo-inositol and agar (8g/L). The media pH was adjusted to 5.8 and autoclaved at 121°C(101kpa) for 15 minutes. The media was poured in pre-sterilized culture tubes (150×25 mm) and incubated in culture room. The media was visualized for any contaminant growth after 3 days.

## **Explant collection and initial treatment**

The procured lateral buds were collected in a jam bottle filled with autoclaved de-ionized water and washed till complete removal of glue-like white discharge. The explants were incubated in 0.5% Ca(OCl)<sub>2</sub> solution

containing two to three drops of each Triton-X and Tween 20 (Hi-Media). After 30 min, washing was carried with autoclaved de-ionized water till complete amputation of detergent traces. The explants were transferred in 0.1% polyvinyl pyrrolidone (PVP) solution till further sterilant treatment (Fig 2a).

### **Sterilant treatment and explant preparation**

The explants were treated with various sterilants for different time intervals *viz.* 0.01% Bavistin™ (5, 10, 15 and 20 min), 0.1% Mercuric chloride (1, 2, 3 and 4 min), 4% Sodium hypochlorite (5, 10, 15 and 20 min) and 70% Ethanol (Table 1). Explants were washed three times with autoclaved de-ionized water (Fig. 2b). Finally, explants were excised in aseptic manner minimizing any damage to the apical part of bud with the help of needed point forceps and scalpel blade in 0.5 cm diameter (Fig 2c). The prepared explants were carefully inoculated on medium (Fig 2d) without touching the walls of culture tube and incubated under 1500 lux white fluorescent light. The growth room conditions were maintained at 16 h of light and 8 h of dark photoperiod, 50-60% RH and 25±2°C temperature.

### **Statistical analysis**

The culture progress was visualized at regular interval of 3 days for contaminant growth for four weeks and data was noted for contaminated, survived and culture growth pattern. Culture survival percent was calculated as per the formula discussed by Kumar *et al.*, (2019). ANOVA was performed and represented as mean±SE, and significant difference was determined at probability level of 5%.

### **Results and Discussion**

The success of in-vitro culture subsistence is

the dependent of explant choice and its source following their sterilization procedures. The field based explant sources face countless contaminants on its exterior. In-vitro propagation of papaya is difficult due to various problems such as: microbial contamination by endogenous bacteria particularly, when field grown plants are used for explant source (Al-Shara *et al.*, 2018; Wu *et al.*, 2012). Moreover, culture media used for in-vitro propagation is the most appropriate for contaminants development thus, complete extrusion or surface disinfection of procured explant is much imperative (Kumar *et al.*, 2019). Under present study, we also faced similar difficulties while cultured lateral buds. Therefore, different sterilant for varying durations had been optimized for higher culture survival.

Under study, the observations noted are given in Table 1 and Fig 3. The control (no sterilant) was observed with no shoot or zero explant survival. Bavistin™ is a widely used fungicide as sterilant in plant tissue culture studies (Khatun *et al.*, 2016). The higher explant survival percent (38.7±0.02) with healthy and uniform growth was noted when explant treated with Bavistin for 10 min as compared to other time durations. Whereas, Bavistin treatment for 20 min resulted in least survival percent and non-uniform shoot growth. The findings under study are in harmony with Kumar *et al.*, (2019).

Mercuric chloride (HgCl<sub>2</sub>) is the chemical compound that is used as disinfectant (Anburaj *et al.*, 2011). Under study, explants treated with 0.1% HgCl<sub>2</sub> for 2 min were found to be healthy and uniform with maximal subsistence percent (40.5±0.9). The explant treated till 4 min with HgCl<sub>2</sub> showed in tissue necrosis. The results observed under study had supported by the study of Rayavalad *et al.*, (2019), whereas Roy *et al.*, (2012) described

HgCl<sub>2</sub> treatment for 8 min and Bindu and Podikunju (2017) conveyed 0.08% HgCl<sub>2</sub> treatment for 12 min while using lateral buds of papaya. Kumar *et al.*, (2019) also reported optimized explant survival with 0.1% HgCl<sub>2</sub> for 5 min for sugarcane apical shoot

meristem. It was noted that increase in treatment duration was led to tissue necrosis and growth was impaired. The bleaching action of two chloride ions that associate strongly with proteins cause tissue necrosis.

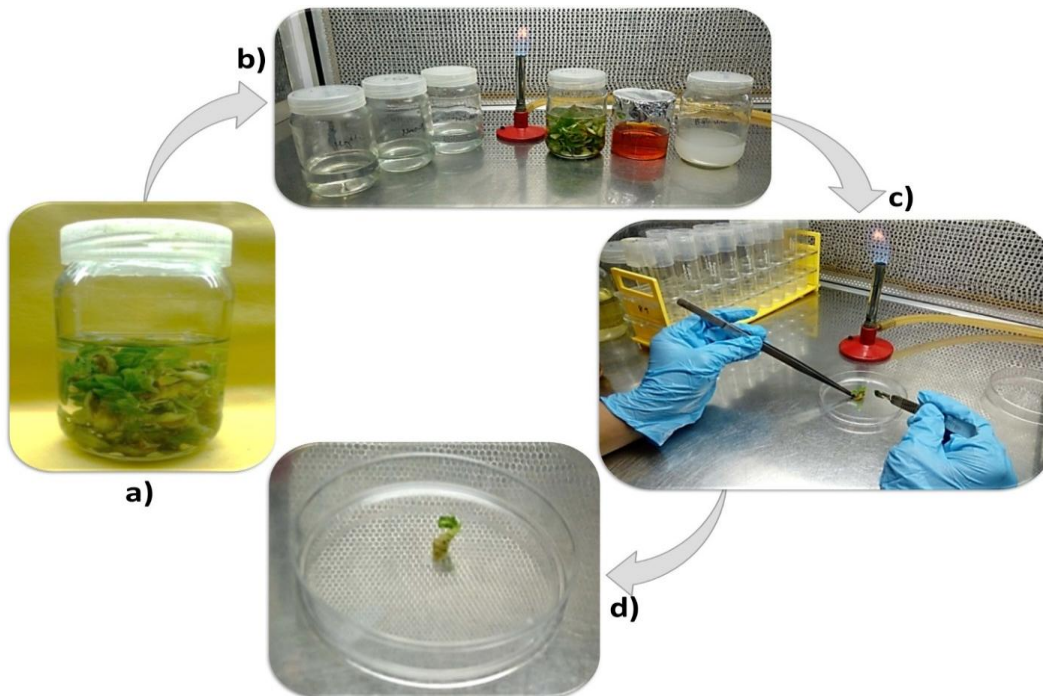
**Table.1** Effect of sterilants on explant survival and growth pattern

Sterilant Conc <sup>n</sup> (%)	Time (min)	Survival %	CV%	Characteristics of shoots
<b>Control</b>		NIL	0	Contaminated
<b>Bavistin (0.1%)</b>	5	35.3 ± 1.3	0.04	Uniform
	10	38.7 ± 0.0	0.02	Healthy and uniform
	15	33.8 ± 1.3	0.04	Uniform
	20	27.0 ± 1.1	0.04	Non- uniform and unhealthy
<b>Mercuric Chloride (0.1%)</b>	1	37.0 ± 1.8	0.05	Uniform
	2	40.5 ± 0.9	0.02	Healthy and uniform
	3	26.0 ± 1.3	0.05	Non-uniform
	4	17.7 ± 1.9	0.11	No shoot induction (Tissue necrosis)
<b>Sodium Hypochlorite (4%)</b>	5	39 ± 0.7	0.02	Healthy and uniform
	10	35.2 ± 1.2	0.03	Uniform
	15	33.7 ± 1.7	0.05	Medium and Uniform
	20	25.3 ± 1.7	0.07	Uniform
<b>Ethanol (70%)</b>	1	37.8 ± 0.6	0.02	Healthy but non-uniform
	2	34.2 ± 0.9	0.03	Uniform
	3	32.0 ± 1.4	0.04	Uniform
	4	29.2 ± 1.2	0.04	Uniform
<b>Combination [Bavistin (0.1%) + HgCl<sub>2</sub> (0.1%) + NaOCl (4%) + EtOH (70%)]</b>	(10 + 2 + 5 + 1) followed by three times washing after each sterilant treatment	46.3 ± 1.5	0.03	Healthy and uniform

**Fig.1** Explant collection from field

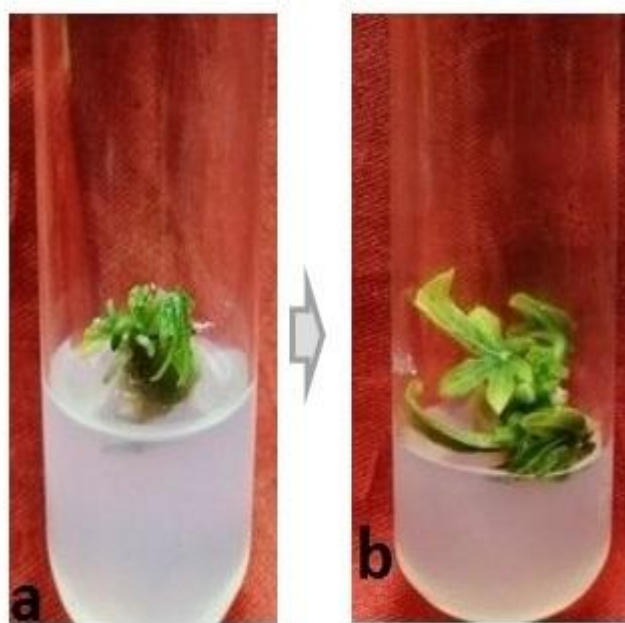


**Fig.2** Explant sterilization; Explant pretreatment (incubation in  $\text{Ca}(\text{OCl})_2$  + Triton-X + Tween 20 a), Sterilant treatment and washing b), Explant preparation c), Explant inoculation d)





**Fig.3** Observations for shoot survival



Sodium hypochlorite (NaOCl) is an effectively used sterilant in micropropagation studies (Kumar *et al.*, 2019). Under study, the subsistence proportion with 4% NaOCl ranged  $25.3 \pm 1.7$  (20 min) to  $39 \pm 0.73$  (5 min). Reda *et al.*, (2004) also considered NaOCl as a potent sterilizing agent for explant surface sterilization.

Ethanol (EtOH) has higher bactericidal action in comparison to other surfactants (Bloomfield, 1978). However, in present study EtOH was not found so much effective as other sterilants, as it showed non-uniform shoot growth. Higher culture survival percent with healthy and uniform shoot growth ( $37.8 \pm 0.6$ ) as compared to other treatments was observed in explants treated with 70% EtOH for 1 min. Our study reports were found in agreement to the study done by Roy *et al.*, (2012).

Under study, the highest shoot survival ( $46.3 \pm 1.5$ ) with optimum shoot growth (Fig. 3) was observed when explants treated in combination by following three washings

with autoclaved de-ionized water after each sterilant treatment. Kumar *et al.*, (2019) also described satisfactory explant sterilization using sterilant combination.

It is concluded under study, lesser treatment duration was showed with higher culture survival but at same time it was not effective to completely removal of bacterial infection. However, increasing duration resulted in tissue necrosis. The satisfactory observations were recorded when explants treated with the sterilant combination.

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