The 9th CASEE 2018 Conference
'Research based education at life science universities in the Danube region towards a sustainable future' București, Romania

IN VITRO EFFECT OF VARIOUS STERILIZATION TECHNIQUES ON PEACH (*Prunus persica* (L.) Batsch) EXPLANTS

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Keywords: *shoot-tip, node, sodium hypochlorite, hydrogen peroxide, boric acid*

Introduction

Peach (*Prunus persica* L. Batsch) is one of the most important stone fruits which are grown extensively in different parts of the world. Peaches are native to China and their planting dates refer to at least 4000 years (Wang and Zhuang 2001). Tissue culture techniques are used for commercial and research purposes extensively to grow many different plants (Hussain et al, 2012). Aseptic conditions are usually practiced in micropropagation, plant tissues on their surfaces inherently have various bacteria and fungi, moulds etc. It is necessary that the explants be free from any surface contaminants prior to tissue culture since contaminants can grow in the culture medium, rendering the culture non activity (Hiremath, 2006). There is so much pathogen (microbial contaminants) which has been a major threat to tissue cultures due to their rapid proliferation characteristics, microbes can be come from explants, laboratory instruments, conditions in the laboratory and contaminants may be introduced with the staff during manipulations in the laboratory (Leifert and Cassells, 2001; Enjalric et al., 1998). Contamination is a real problem that opposes the progress and development of tissue culture technology (Webster et al., 2003). These microbes compete adversely with explants for nutrients, and their presence often results in variable growth or increased culture mortality or can also result, reduced shoot proliferation, tissue necrosis and reduced rooting (Oyebanji et al., 2009). A successful *in vetro* culture protocol, starts with effective explants sterilization, the sterilization chosen for an experiment depend on the type of explants, Sterilize material and plant genotype (Dodds and Roberts, 1985; Rezadost et al., 2013). There are several various sterilization factors are used to sterilize tissues, these disinfect materials are also toxic to explants tissues, and therefore select the correct concentration and the times of exposure to explants, must be elected to reduce the injury of plants (CPRI, 1992).So there is a state of balance between sterilizing explants and killing the explants themselves (Qin et al., 2012 and Olewet al., 2014). Many researchers have used these sterilizing agents successfully; also there are studies on the effect of fungicides and antibiotics on these kinds of contaminants (George, 1993; Rashid et al., 2008; Maqbool et al., 2010; Bakhsh et al., 2012). Several different mechanism are used to eliminate fungal and bacterial contamination, including the use of inactivation by heat and light, fungicides and antibiotics, the time of sterilization is dependent on the type of tissue (Haldeman et al., 1987; Kneifel and Leonhardt, 1992; Leifert et al., 1992). Explants are commonly surface-sterilized using ethanol, sodium hypochlorite, mercuric chloride, hydrogen peroxide, fungicides and antibiotics. Therefore, the present study was conducted to compare different sterilizing protocols for peach micropropagation and to find out the best, efficient and cost effective sterilization procedure that may result in least or no contamination in peach tissue culture. In our study we compared several modifications of four surface sterilization methods based on the use of, sodium hypochlorite, hydrogen peroxide, captan (50%) and boric acid with using explants of peach accessions with different degrees of contamination.

Materials and Methods

Shoot tips and nodes (0.5–1 cm in size) of peach (*Prunus persica* L. Batsch cv Florin) were collected from the orchard of trees planted in the field of experiments to the Faculty of Horticulture University of Agricultural Sciences and Veterinary Medicine in Bucharest to used as explants for *in vitro* culture establishment. Explants were placed under running tap water with detergent for 30 min to remove any foreign contaminants. After washing, explants were dissected and surface sterilized in a laminar air flow hood with rinsed with ethanol 70% for 2-3 min then were washed with distilled water three times for 2-3 min after that, For peach tissue culture initiation, four sterilization agents where tested in 18 different variants: Sodium hypochlorite (NaOCl) in three concentrations: 5%,10% and15%, for 5 and 10 min; Hydrogen peroxide (H_2O_2), in two concentrations: 5% and 10%, for10 and 20 min; Captan (50%) WP fungicide, in four concentrations: 1%, 2%, 3% and 4%, for 5 min and Boric acid ($B(OH)_3$) in two concentrations: 1% and 2%, for 5 and 10 min. (table 1).The explants (shoots-tips and nodes) were cultured in MS (Murashige and Skoog., 1962) basal medium supplemented with 30 g sucrose, as carbon source and 7 g agar. pH was adjusted between 5.7 and 5.8 by using either 1N HCl or 1N NaOH before the agar was added. Media was then heated on a hot plate with continuous stirring using a magnetic stirrer until agar is dissolved and media put in the culture tubes. The culture tubes were covered with lids and put in trays and autoclaved. Autoclave was adjusted at a temperature of 121°C for 15 min. The growth chamber for the *in vitro* cultures had 22±2°C temperature and 80-85% relative humidity, with a photoperiod of 16 h day light and 8h dark. (Stănică et al. 2002). In the experiment ten replicates (one explants in one tube culture) were used for each treatment and the experiment was repeated twice.

Tables and pictures

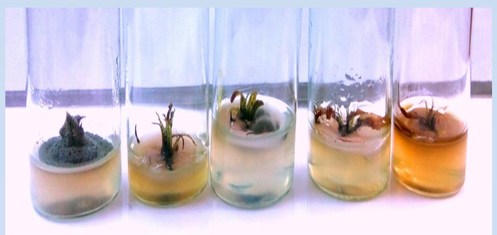

Fig 1. Fungal contamination on peach explants after 14 day from sterilization V9 (H_2O_2 10% for 10 min)

Fig 2. Fungal contamination on peach explants after 14 day from sterilization V11 (captan 1% for 5 min)

Table 1. Effect of surface sterilizer, various concentrations and time exposure on % of contamination and % of survived on explants after 14, 28 days from culture.

Variants	Surface sterilizer	Concentration (%)	Exposure time (min)	Shoot-tips			Nodes		
				Contamination (%)		Survived (%)	Contamination (%)		Survived (%)
				after 14 days	after 28 days		after 14 days	after 28 days	
V1	Sodium hypochlorite	5	5	70	100	00	80	100	00
V2	Sodium hypochlorite	5	10	50	90	10	70	100	00
V3	Sodium hypochlorite	10	5	45	70	30	50	70	30
V4	Sodium hypochlorite	10	10	20	40	60	35	40	60
V5	Sodium hypochlorite	15	5	25	50	50	40	50	50
V6	Sodium hypochlorite	15	10	50	70	30	70	80	20
V7	Hydrogen peroxide	5	10	70	85	15	70	85	15
V8	Hydrogen peroxide	5	20	65	85	15	65	85	15
V9	Hydrogen peroxide	10	10	65	80	20	65	80	20
V10	Hydrogen peroxide	10	20	50	75	25	60	80	20
V11	Captan 50%	1	5	35	90	10	50	100	00
V12	Captan 50%	2	5	35	90	10	70	100	00
V13	Captan 50%	3	5	30	80	20	40	85	15
V14	Captan 50%	4	5	30	75	25	45	85	15
V15	Boric acid	1	5	50	100	00	70	100	00
V16	Boric acid	1	10	50	85	15	60	100	00
V17	Boric acid	2	5	40	80	20	70	90	10
V18	Boric acid	2	10	45	80	20	80	85	15

Table 2. Effect of surface sterilizer, various concentrations and time exposure on % of contamination (fungus+ bacteria and sterilizers) on explants after 28 days from culture.

Variants	Surface sterilizer	Concentration (%)	Exposure time (min)	Shoot-tips			Nodes		
				Contamination (%)			Contamination (%)		
				after 28 days	fungus+ bacteria	sterilizer	after 28 days	fungus+ bacteria	sterilizer
V1	Sodium hypochlorite	5	5	100	100.00	00.00	100	100.00	00.00
V2	Sodium hypochlorite	5	10	90	90.00	00.00	100	100.00	00.00
V3	Sodium hypochlorite	10	5	70	59.50	10.50	70	63.00	7.00
V4	Sodium hypochlorite	10	10	40	30.00	10.00	40	36.00	4.00
V5	Sodium hypochlorite	15	5	50	15.00	35.00	50	42.50	7.50
V6	Sodium hypochlorite	15	10	70	30.00	40.00	80	24.00	56.00
V7	Hydrogen peroxide	5	10	85	85.00	00.00	85	85.00	00.00
V8	Hydrogen peroxide	5	20	85	85.00	00.00	85	85.00	00.00
V9	Hydrogen peroxide	10	10	80	64.00	16.00	80	72.00	8.00
V10	Hydrogen peroxide	10	20	75	52.50	22.50	80	68.00	12.00
V11	Captan 50%	1	5	90	90.00	00.00	100	100.00	00.00
V12	Captan 50%	2	5	90	90.00	00.00	100	100.00	00.00
V13	Captan 50%	3	5	80	56.25	23.75	85	76.50	8.50
V14	Captan 50%	4	5	75	56.25	18.75	85	67.25	12.75
V15	Boric acid	1	5	100	100.00	00.00	100	100.00	00.00
V16	Boric acid	1	10	85	85.00	00.00	100	100.00	00.00
V17	Boric acid	2	5	80	60.00	20.00	90	65.50	4.50
V18	Boric acid	2	10	80	60.00	20.00	85	67.25	12.75

Table 3. Effect of surface sterilizer, various concentrations and time exposure on mean shoots length (cm) and mean (no) leaves shoot on explants after 28 days from culture.

Variants	Surface sterilizer	Concentration %	Exposure time (min)	Shoot-tips			Nodes		
				Survived after 28 %	Mean shoot length (cm)	Mean shoot Leaves (on)	Survived after 28 %	Mean shoots length (cm)	Mean shoot Leaves (on)
V1	Sodium hypochlorite	5	5	00	0.00	0.00	00	0.00	0.00
V2	Sodium hypochlorite	5	10	10	4.64	5.70	00	3.12	7.88
V3	Sodium hypochlorite	10	5	30	3.32	4.22	30	3.12	7.10
V4	Sodium hypochlorite	10	10	60	3.17	4.05	60	2.78	5.87
V5	Sodium hypochlorite	15	5	50	3.25	3.50	50	2.55	4.65
V6	Sodium hypochlorite	15	10	30	2.64	3.12	20	2.06	4.50
V7	Hydrogen peroxide	5	10	15	4.34	4.44	10	3.45	7.33
V8	Hydrogen peroxide	5	20	15	3.89	4.21	10	3.12	7.02
V9	Hydrogen peroxide	10	10	20	3.11	4.00	20	3.00	5.16
V10	Hydrogen peroxide	10	20	25	2.64	3.66	20	2.77	5.00
V11	Captan 50%	1	5	10	4.50	5.99	00	0.00	0.00
V12	Captan 50%	2	5	10	4.22	5.78	00	3.13	7.23
V13	Captan 50%	3	5	20	3.80	4.43	15	2.98	7.11
V14	Captan 50%	4	5	25	3.62	3.68	15	2.77	6.78
V15	Boric acid	1	5	00	0.00	0.00	00	0.00	0.00
V16	Boric acid	1	10	15	4.17	4.16	00	0.00	0.00
V17	Boric acid	2	5	20	3.87	4.20	10	3.01	6.89
V18	Boric acid	2	10	20	3.45	3.80	15	2.48	6.22

Results and Discussion

EFFECT OF STERILIZATION FACTORS

The study showed there is an effect of substances used in sterilization, sodium hypochlorite the most effective treatment with 50% survival rate in 15 % for 5 min and 60% in 10 % for 10 min and has outperformed the rest of the other sterilizers which their results were not satisfactory as the results were hydrogen peroxide (H_2O_2) with 25 % survival rate in 10 % for 20 min; Captan 50 % with 25 % survival rate in 4 % for 5 min; Boric acid ($B(OH)_3$) with 20 % survival rate in 2 % for 10 min (table 2). These results are similar to the studies Satish et al. (2012) on sugarcane; Siddique et al. (2018) on *Skimmia laureola*, when they used different substances in sterilization, where the results differed according to the sterilizers. Studied by many researchers, the solution of sodium hypochlorite for superficial sterilization of explant was efficient and didn't injury the explants at appropriate focus (Gertlowski K. and Petersen M., 1993). These results are similar with those of Hippolyte. (2000), which referencethat the high focus of sodium hypochlorite can be effective in sterilizing the superficial explants cultivated *in vitro*, but it is accompanied by the death of explants. Many researchers have found these sterilizing agents successfully (Rashid et al., 2008; Maqbool et al., 2010; Bakhsh et al., 2012).

EFFECT OF CONCENTRATION AND EXPOSURE TIME

The study showed that there was a correlation between the dipping period and the concentration of the substance used in the sterilization on the extent of their effect on the percentage of explants survived, explants contaminated by fungi, bacteria and explants dead due to the increased concentration of the material used (tables 1, 2 and 3). Increasing the exposure duration and sterile concentration had reduced the contamination rate but highest number of loss explants resulted. The influence of sterilizing chemical ruin the shape and functions of microbe's enzymes (George et al., 2008). But the increasing exposure duration and concentration of sterilizes above certain optimum limit cause loss of explants because of the oxidant chemical ingredient ruin the plant tissue as well (Danso et al., 2011).

These findings are similar of the negative effects of Sodium hypochloride at high concentration were observed (Colgecen et al., 2011). And a higher concentration of hydrogen peroxide 5% was reported to negatively affect in sunflower (Dolatabadian and Modarressanavy., 2008).

EFFECT OF EXPLANTS

The study showed there are differences in the extent of the response of the explants used in rate of plant growth (shoots length and leaves number), contaminated rate and survival rate (table 2 and 3). also, the shoots had been registered the lowest rate of infection and the most effect to increase the concentration of sterile material compared to the contract also explained that the shoots and nodes gave the best rate of shoots length and leaves number formed when using concentrations less. These results are similar with (Rezadost et al, 2013) who confirmed that the surface sterilize used for an experiment typically depend on the explants and plant species.

Conclusion

Among the different sterilization protocols tested for the successful establishment of *in vitro* culture of peach tissue culture. Our results showed that during the sterilization were different depend on the sterilization factors, exposure time and explants type was used for micro- propagation. It is recommended for this study to be used among the different sterilization variants, sodium hypochlorite was the most effective treatment with 50% survival rate at V5 (15% for 5 min) and 60% survival rate at V4 (10% for 10 min). Recommended also hydrogen peroxide (H_2O_2) 10 % for 20 min; Captan 50 % 4 % for 5 min; Boric acid ($B(OH)_3$) 2 % for 10 min recommended to use in the initial sterilization.

Acknowledgements

The authors want to thanks all their colleagues, students researchers, management and staff in micropropagation laboratory, Centre for Studies of Food and Agricultural Products Quality, USAMV for their assistance in this research activity.

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