IN VITRO PROPAGATION OF *PRUNUS LAUROCERASUS* L.

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Abstract

The study purpose was to initiate Prunus laurocerasus Novita in vitro cultures from minicuttings and in vitro rootedness process stimulation with activated charcoal. Initiating the in vitro culture plant heavily depends by the donor plant health, respectively by its endogenous contamination with various microorganisms. Prunus laurocerasus initiation from minicuttings was successful on MB-MS + G, solidified culture medium, with 0.1 mg / l IBA and 2 mg / l BA and optimal time for sodium hypochlorite 5% plus Tween 20 disinfections was 12 minutes. Explants provided from polluted with dust (roadside - curtains of dust) area had endogenous infections and were impossible to initiated in vitro, compared with explants from an unpolluted area (protected). A fact still unclear occurred on MS medium with added charcoal 2%, namely changing the pH value from 5.7 to 8. We suppose that change favoured the growth of certain bacteria (already in the growth chamber), and the entire culture of this experimental lot loss. In the control group, with no activated charcoal, the pH was maintained at levels similar to those of the initial and bacterial infections have not been shown. We intend to continue research on pH modification during in vitro culture and identify bacterial species.

Key words: *Prunus laurocerasus*, micropropagation, explant, activated charcoal, infections

INTRODUCTION

Prunus laurocerasus belongs to the *Rosaceae* family and is a species with an important ornamental interest, but is also grown for its fruit that can be eaten fresh, dried or processed and is used in traditional medicine in various medical purposes (Akbulut et al., 2007; Sahan, 2011; Sulusoglu, 2012, 2014; Turan et al., 2013; Sayinci et al., 2015; Sulusoglu et al., 2015), including diabetes (Orhan et al., 2015). With a relatively fast growth, temperature resistance from -15°C in winter, the leaf permanently green, in recent years this species has become increasingly sought on the outdoor ornamental plants market. The plant can form hedges, but is aesthetically arranged solitary.

In vitro cultures are a quick and efficient instrument with possibilities to improve this species, slightly pretentious to temperature and contributed to the improvement and conservation of *Prunus* (Martínez-Gomez et al., 2005; Islam, Vardal, 2009; Sulusoglu, Cavusoglu, 2010, 2013; Cheong, 2012; Sadeghi et al., 2015). It was resorted to studying explant choice (pollen inclusive) (Sulusoglu, 2014; Sulusoglu, Cavusoglu, 2014 a, b) either to optimize the culture medium composition from *Prunus*, research in this area are many and continues to attract the researchers interest.

Sulusoglu (2012) improved *in vitro* production *Prunus laurocerasus* plantlets on MS medium with 2.0 + 0.5 mg / l BA + IBA but the most optimal way to stimulate growth and subsequent acclimation *ex vitro* plantlets was that adding hormonal balance into a certain growth stage, ie after 60 days. A year later, Sulusoglu and Cavusoglu (2013) showed that the best developed laurel roots was with 0.5 mg / l IBA addition in the medium MS, the percentage of *ex vitro* survival being amounted to 77.3%. In this paper we intend to identify other micropropagation peculiarities in this species.

MATERIAL AND METHOD

The plant material consisted of *Prunus laurocerasus* Novita minicuttings with two nodes, derived from plants which was in the second year of growth in the field, in two different environments conditions: devoid of urban pollution or car traffic and a polluted environment, in the vicinity of a road, serving as a curtain of dust on its edge.

Branches of 10-15 cm were taken from the donor plants and were brought to the plant biotechnology laboratory where they were subjected to micropropagation procedures (Table 1). Research was conducted in June 2015 - May 2016 period.

Table 1

Research design

In vitro culture initiation			
Explant type	minicuttings with two nodes, derived from two different locations: unpolluted and polluted		
Sterilization	Alcohol 70% - few seconds submersion Sodium hypochlorite 5% + Tween 20 for 5, 12, 20 minutes Clean with sterile water – 25 minutes		
Culture medium	Basal, solidified MB-MS+G, with 0,1 mg/l IBA and 2 mg/l BA		
Culture recipients	Uncoloured glass recipients, with 11 cm height and 2 cm diameter		
In vitro growth conditions	1700 lx, 16/24 h light, 24 – 25 ˚C		
Culture period	1 month		
In vitro rooting			
Explant type	minicuttings with two nodes		
Experimental variants /substratum type	V_0- MB-MS+G, without AC and growth regulators (*control*)	V_1- MB-MS+G, plus AC 2%	V_2- MB-MS+G plus AC 2% + 0,1 mg/l IBA and 2 mg/l BA
Culture recipients	Uncoloured glass recipients, 11/2 cm		
In vitro growth conditions	1700 lx, 16/24 h light, 24 – 25 ˚C		
Culture period	3 months		

NOTE: MB-MS – Basal Murashige-Skoog (1962) medium; G – Gamborg et al. (1968) vitamins; IBA – indole-3-butiric acid; BA – N^6-benzyladenine; AC – activated charcoal.

Cuc and Petruş-Vancea (2016) reported that the stem and leaf *Prunus laurocerasus* anatomical structure lends itself to be easily disinfected to be used in *in vitro* culture initiation, provided they do not possess endogenous infections.

RESULTS AND DISCUSSION

Explants from the polluted area were infected at lots with 5 and 12 minutes hypochlorite submersions (Fig. 1) and at 20 minutes disinfected lot, although infections were missing, but explants were necrotic because of the disinfectant agent that acted too long, damaging cells, all percentage of initiation was zero (Fig. 2). The fact that infections occurred in 1 month after culture initiation, then develop either the upper part or around of the explant), confirmed that they are endogenous and not a faulty disinfectant or inoculation technique. Such infections caused by human error, occurring in 3-5 days after the inoculation.

The time of disinfection plant material regardless - in order to initiate sterile culture - endogenous infections with bacteria or fungi that are already in the tissue donor plant cannot be eliminated. One solution would be the use of thermotherapy on donor plants and initiate *in vitro* culture from apical meristems explants.

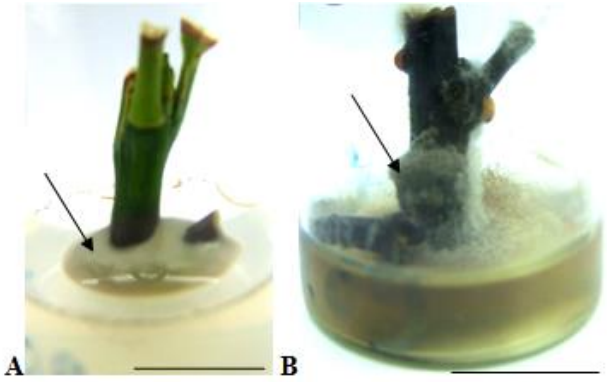

Fig. 1. The infections emergence (arrows) at *Prunus laurocerasus in vitro* explants provided from polluted area caused by bacteria (A) and fungi (B) (bars means 1 cm)

At explants derived from the unpolluted area, the regeneration was 67%, when sodium hypochlorite sterilization took 12 minutes (Fig. 2) or to the basal node (Fig. 3 A), or the upper node (Fig. 3 B) or at both nodes (Fig. 3 C), when the basal node was just ½ submerged in the culture medium.

In this experiment the callus formation was not wanted, it was revealed, but it did not affect the objectives of the regeneration experiment because the callus was formed mainly at internodes level (Fig. 3 C).

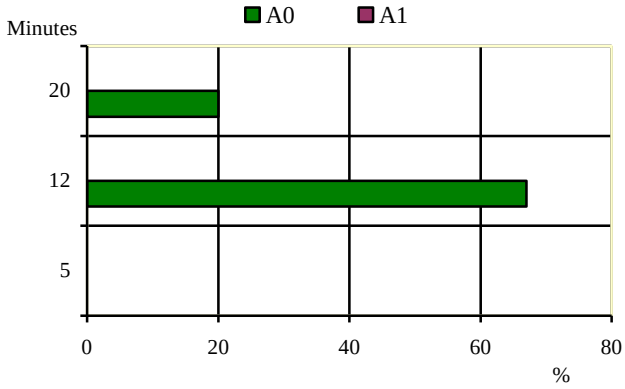

Fig. 2. *Prunus laurocerasus in vitro* regeneration percentage, 1 month after culture initiation, depending on the explant origin: A_0 - the unpolluted area and A1 - polluted area, and during submersion in disinfectant, where 100% is the number of explants initially inoculated

At 1 month from initiation, the regenerated plant had 0.6 stem length average, 2 – 3 nodes and 3-4 leaflets number average (Table 2).

Table 2

Prunus laurocerasus plantlets growth indices at 1 month from *in vitro* initiation

Measurements	**Stem length (cm)**	**Nodes no.**	**Leaves no.**
Average ±standard deviation	0.62±0.72	2.96±0.78	3.62±0.58

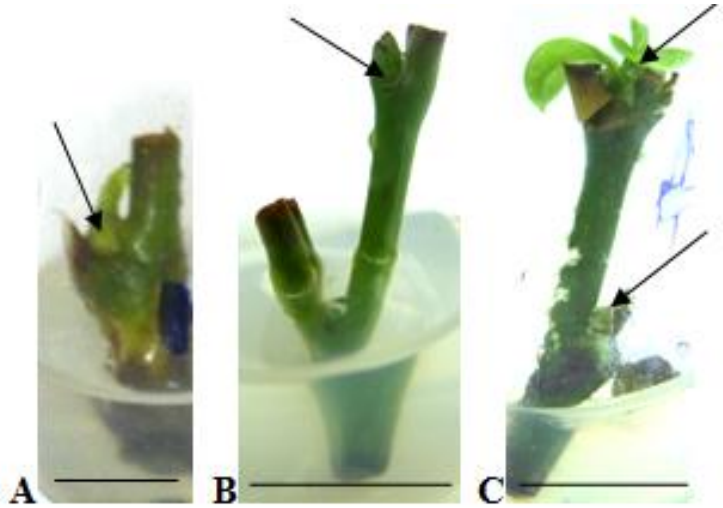

Fig. 3. *Prunus laurocerasus* regeneration (arrows), at 1 month from *in vitro* initiation, on basal node (A), on upper node (B) or both nodes (C) (bars means 1 cm)

The subculture was made on mediums with and without activated charcoal (Fig. 4-5).

In previous research (Petruş-Vancea et al., 2004), on *Petunia* cultivated *in vitro*, the culture medium variants with activated charcoal added generated plantlets whose growth indices showed increases than those provided from similar conditions but without activated charcoal.

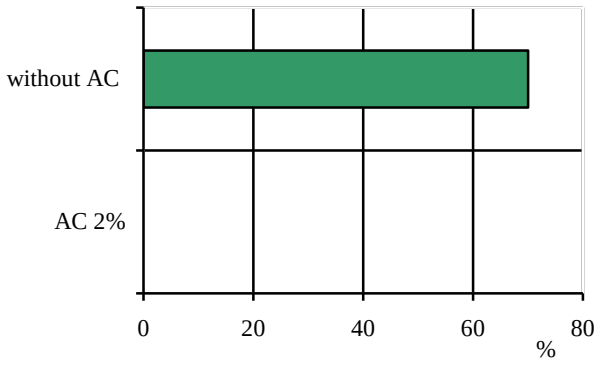

Fig. 4. *Prunus laurocerasus in vitro* regeneration percentage, 3 months after subculture on basal MB-MS+G, medium without activated charcoal (AC) and with activated charcoal 2%, where 100% is the number of initially inoculs

In this experiment, at *Prunus laurocerasus* we met a special case, namely: only on medium with activated charcoal 2% additions, in an advanced stage of its completion, respectively at 3 months of subculture, had infections caused by a bacterium (after macroscopic specific aspect) and culture died (Fig. 4). The presence of any kind of infections (bacterial or fungal) in the culture media is a fact undesirable, resulting in culture loss, except in cases in which the co-culture studies among such microorganisms and explant (Cachiță et al., 2008; Petruş-Vancea et al., 2011), strictly for purposes of fundamental research.

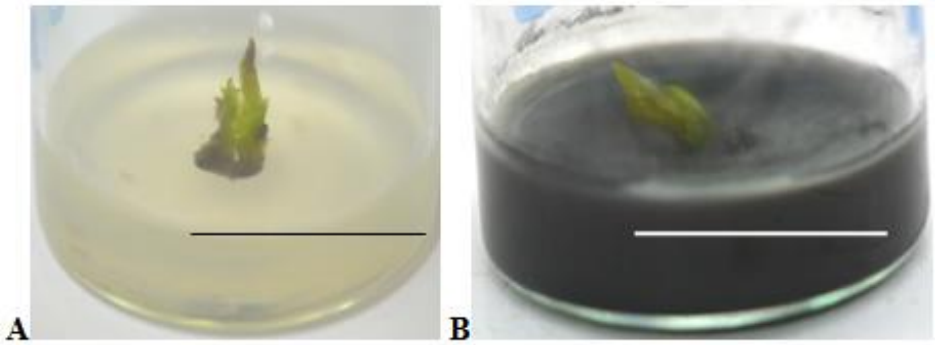

Fig. 5. *Prunus laurocerasus* subculture, at 1 month on basal MB-MS+G, medium without activated charcoal (A) and with activated charcoal (B) (bars means 1 cm)

In our case, that infection occurred only after three months of subculture suggests that there was not an inoculation error. It also cannot question the existence of endogenous infections because phytoinoculs comes from a sterile subculture. The question is: what is in the containers with added charcoal which favoured this infection? What are the physical or chemical factors that have created the optimum conditions to develop these bacteria? To answer such a question would require extensive research, multidisciplinary. For starters, we plan to realize some culture medium pH measurements, at 3 months of subculture. Measuring the pH of the medium with charcoal, he presented 8 values, up from 5.7. We suppose that this situation has created favorable conditions for bacteria growth, which is not found on activated carbon-free medium (which maintained pH close to 5.7). Hence our conclusion was that: the bacteria that caused the infection at 3 months of secondary subculture comes from the growth room, but was itself only with the addition of activated carbon media due to changes in pH. An identical situation we mentioned in another experiment with *Vaccinium*, made in the same period (own data).

In this regards, we have identified research literature confirming that sometimes the activated charcoal presence in the culture medium can alter pH, even immediately after medium autoclaving (Owen et al., 1991). Due to the different anions and cations pytoinoculs absorption in the culture medium, its pH does not remain constant (George et al., 2008). Stasinopoulos and Hangarter (1990) stated that photochemical changes can occur also in culture media. Such pH can be influenced by the temperature of the growth room, maintaining the dark or light. Store in the dark at 4°C was found to reduce medium acidification, compared with the storage at the standard temperature (25°C light), regardless of the gelling agent used, the type of activated carbon or the storage period.

CONCLUSIONS

1. *Prunus laurocerasus in vitro* culture initiation from minicuttings was successful on MB-MS + G, solidified culture medium with 0.1 mg / l IBA and 2 mg / l BA and optimum time for keeping the cuttings in sodium hypochlorite being for 12 minutes.
2. In *in vitro* culture initiation is extremely important source of explant donor material. Explants from areas polluted with dust (roadside - curtains of dust) had endogenous infections and were impossible to use for *in vitro* initiating compared with explants from an unpolluted area (protected).
3. Activated charcoal presence in culture medium in our research on *Prunus laurocerasus* was not beneficial because of the induced change

in medium pH during *in vitro* culture, resulting in microorganisms' overgrowth and culture loss.

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