RESEARCH ARTICLE **(Open Access)**

Optimization of explants sterilizations intended for In vitro culture of sp. *Prunus avium*

AVDIRRAHMAN GASHI[1*], TOKLI THOMAJ[1] , TAULANT MITRUSHI[2], EDLIRA KUKALI[1]

[1]Faculty of Agricultural & Environment, Agricultural University of Tirana &Faculty of Agricultural & Environment, Agricultural University of Tirana,

[2]In Vitro laboratory Elbasan.

*Corresponding author E-mail: avdi-gashi773@hotmail.com

Abstract

The aim of research was establishment and maintenance of in vitro explants free from bacteria. *PrunusAvium*explants intended for tissue culture as object of research treated with different concentrations of sterilizante. The presence of bacterial contaminants is extremely important to micro propagate plants.

Different concentration of $HgCl_2$ (0.01; 0.02 %) and NaOCl (0.25; 0.5%) for varying exposure time (15; 20 min).The highest contamination (80%) and least explants survival were identification when used 0.02% $HgCl_2$ or 90 % with 0.25%NaOCl.

Minimum death of explants was in 0.01% $HgCl_2$ and 0.5 % NaOCl disinfected for 15 min respectively 90%-95%.Isolated buds, after sterilization with 0.01% HgCl2 for 15 min are characterized by higher survival rate, respectively 85% for *P. mahaleb* and 92% for *P. avium* (rootstocks: i - *P. avium x P canascens* (Gisela 6)

Keywords: explants; *prunusavium*; sterilization;in vitro; concentration.

1. Introduction

Recently, breeding practices in Prunus have been advanced by the development and application of micro propagation. Micro- propagation offers the possibility of scale multiplication planting material. Explants surface sterilization is one of the critical steps in micro propagation of plants. Microbial contamination is one of the most serious problems in micro propagation. Contamination with microorganisms is considered to be the simple most important reason for losses during in vitro culture of plants. Such microorganisms include viruses, bacteria, yeast, fungi, etc. These microbes compete adversely with plant tissue cultures for nutrients. The presence of these microbes usually result in increased culture mortality but can also result in variable growth, tissue necrosis, reduced shoot proliferation and reduced rooting, Determination of effective explant surface sterilization procedure is essential to avoid the problem of contamination during in vitro culture.

No single sterilization procedure would do for all the species. Even for the same species or the same variety, a single formula may not work at different time. This is due to the fact that load and type of microorganism on explants is dependent on seasons.

Disinfectants such as ethanol, NaOCl, and Tween 20 hamper the growth rate of fungi and bacteria on the growth media.

Hypochlorite is known to be a very effective killer of bacteria; even micro molar concentrations are enough to reduce bacterial populations significantly. However, little is known about the exact mechanisms of its bactericidal activity. When diluted in water, the hypochlorite salts (NaOCl, Ca (OCl)2) lead to the formation of HOCI whose concentration is correlated with bactericidal activity. Sodium hypochlorite is readily available and can be diluted to proper concentrations. A balance between concentration and time must be determined empirically for each type of explants because of phytotoxicity. Therefore, the aim

of this study was to assess the effective prunus explant- surface sterilization.

2. Material and method

The study was conducted at the Micro propagation Laboratory, of Agricultural University of Tirana from October 2015 to January 2016.

Source and choice of plant materials

The four rootstocks and selected material is presented in this study:

PrunusaviumL.,(Cerasusavium L. var. silvestris Ser., P. cerasus) rootstocks.

a - *P. avium x P canascens* (Gisela 6)

b - *P.avium x P. pseudocerasus*[Colt (nano)] a valuable plant as rootstocks grows in northern areas.

c - *P. mahaleb x P.avium*(Maxma) resistant against phytopathogens

Prunusmahaleb L. –; endangered plants VU (A1b) according to "Read Book";

Actively growing shoots (juvenile plants) were taken as they are more responsive to shoot regeneration and proliferation than shoot explants from adult forms as reported by Charles V. M. Fink [1]

2.1. Explants surface sterilization

Before explants were placed on a medium (inoculated), it must be sterilized to make them free of all microorganisms. The leaves were removed from the explants and soaked in tap water and brought to laboratory. The explants were then thoroughly washed with tap water 3-5 times followed by liquid soap for 30 min with agitation to physically remove most microorganisms. Then the explants were treated with 70% ethanol for 30s under laminar air flow cabinet.[2]

After pretreatment with ethanol, the explants were rinsed with distilled water three times, to lower the toxic effect of ethanol. After decanting the sterilizing solutions under safe condition, the explants were washed three-times each for 5 min with sterile distilled water to remove traces of NaOCl & $HgCl_2$.[4]

They were then treated with levels of concentration of sodium hypochlorite and Dichloromercury (NaOCl and HgCl2), respectively, with 0.5- 0.25%; 0.01-0.02 % over different exposure times (15 and 20 min).[3]

After decanting sterilizing solutions under safe conditions, the explants were washed three times each for 5 min with sterile distilled water to remove traces of densifectantes.

2.2 Data collection and Statistical analysis

The sterilization experiments data recorded include the number of contaminated, dead and survived.

All the experimental data were processed by methodsTukey–KramerANOVA with importance level 95% ($P < 0.05$) and using statistical program JMP 7.0 statistical program.[5]

2.3 Conditions of culture

After labeling on the varieties/plants,the name and type of explants, all plants were placed in the chamber for cultivating the plants, with physical parameters control (temperature 25 ° C ± 2o C, intensity lighting 2000 lux and fotoperiodes 16 hour light/24 hours). For each stage of development, cultures/ held about 4 weeks.[4]

3. Results and Discussion

Analysis of variance (ANOVA) revealed that concentration of bleach and local exposure time, and the interaction effect was very significant difference (PS0.0001) in growth media pollution, death and level survival of explants.

Different concentration of $HgCl_2$ (0.01; 0.02 %) and NaOCl (0.25; 0.5%) for varying exposure time (15;20 min). The highest contamination (80%) and least explants survival were identification when used 0.02% $HgCl_2$ or 90 % with 0.25% NaOCl.

Minimum death of explants was in 0.01% $HgCl_2$ and 0.5 % NaOCl disinfected for 15 min respectively 90%- 95%. Isolated buds, after sterilization with 0.01% $HgCl_2$ for 15 min are characterized by higher survival rate, respectively

85% for *P. mahaleb* and 92% for *P. avium* (rootstocks: i - *P. aviumx P canascens* (Gisela 6)

Surface sterilization should not kill or break off the biological activity of explants,but the contaminants.[6]

Explants must be surface sterilized only by treatment with disinfectant solution at suitable concentrations for a specified period [7]. Therefore, 0.25%NaOC llocal bleach for 15 min exposure time was found to be the most effective one for explants taken from shoots.

(a)

(b)

Figure 1: Laibeling plant materials (a) and labaratory In Vitro in the Department of Horticulture in the Agriculture University in Tirana(b).

4. Conclusions

•Buds isolation after sterilization 0.01% $HgCl_2$ for 15 min are characterized by higher survival rate, respectively 95% for *P. mahaleb* and 92% for *P. avium* (rootstocks)

•Sodium Hypochlorite Concentration of 0.15 %, 15 min was does result effective in sterilization of explants, isolated from trees on the field.

•I must point out that, the explants are isolated from the trees of the field with high levels of pollution and consequently they needed to sterilization more powerful with reagents such as mercury chloride.

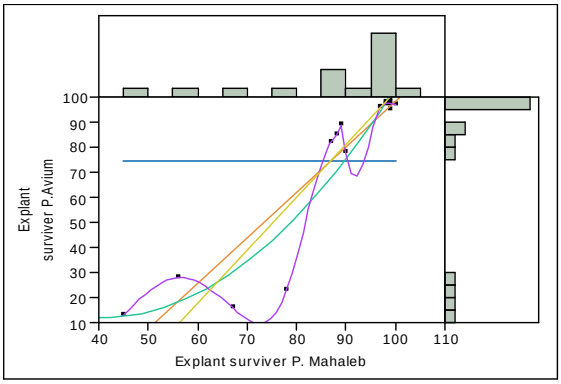
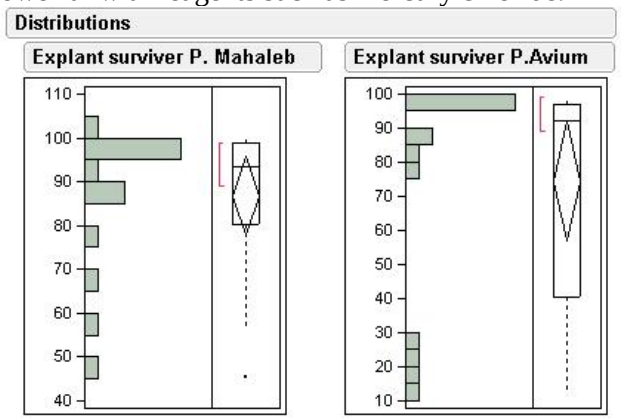
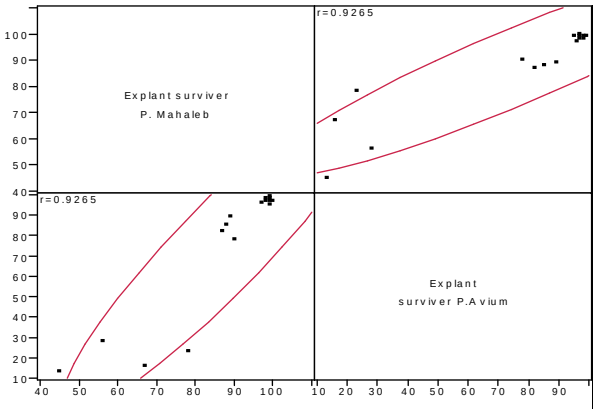


Figure 2. Percentage of survival explantes after sterilizatin with various reagents



(a)



(b)

Figure 3: Distributions of surviver explants(a) and coefficient correlations of surviver explants(b)

Linear Fit
Explant surviver P.Avium = -83.0693 + 1.8143332*Explant surviver P. Mahaleb
Distributions

Table 1: Coefficient of correlations (a) and correlation of explants surviver (b)

(a)

Variable	by Variable	Correlation	Count	Signif Prob	Plot Corr
Explant surviver P.Avium	Explant surviver P. Mahaleb	0.9265	16	<.0001	

(b)

	Explant surviver P. Mahaleb	**Explant surviver P.Avium**
Explant surviver P. Mahaleb	1.0000	0.9265
Explant surviver P.Avium	0.9265	1.0000

5. References

1. Fink CH, Sticklen M, Lineberger D, Domir C: **In vitro organogenesis from shoot tip, internode, and leaf explants of Ulmus x 'Pioneer'**. Plant Science1986, 70(3), 237–245
2. Felek W, Mekibib F and Admassu B: **Optimization of explants surface sterilization condition for field grown peach (*Prunus persica L.* atsch. Cv. *Garnem*) intended for in vitro culture.** African Journal of Biotechnology 2015, 14(8), 657 -660.
3. Paolin A, Trojan D, Carniato A, Tasca F, Massarin E, Tugnoli A, Cogliati E: **Analysis of the effectiveness of Sodium Hypochlorite decontamination of cadaveric human tissues at retrieval**, Springer Netherlands 2016; 17(4), 611–618.
4. Davis Ph: **Lighting: The principles**; 1988:16-26
5. Oehlert G: **A First Course in Design and Analysis of Experiments;** 2000:153-175
6. Alam F, Uddin E, Amin R, Razzak A: **Studies on the Effect of Various Sterilization Procedure for in vitro Seed Germination and Successful Micropropagation of Cucumis sativus**, International Journal of Pure & Applied Bioscence 2006,4 (1), 75-81
7. Ahmad M, Nangyal H, Imran M and Ullah F: **Optimization of Protocol for Surface Sterilization and Callus Induction for Three Rice Varieties**, African American-Eurasian J. Agric. & Environ. Sci 2016, 16 (2), 357-361.