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Control of contamination during micropropagation process of Rootstocks Mariana *(Prunus mariana)*

Saleh Amiri*[1], Shahnam Ashtari[2], Abdullah Hossein Babaiy[3], Samad Aliyoun Nazari[4], Ehsaneh Khodadadi[5], Ehsan Khodadadi[6] and Mohsen Sabzi[5]

[1]*Department of Agricultural Biotechnology, North West and West Biotechnology Institute, Iran*
[2]*Department of Horticulture Science, University of Tabriz, Iran*
[3]*Department of Plant Breeding, Member of Young Researchers Club, Tabriz Branch, Islamic Azad University, Tabriz, Iran*
[4]*Department of Horticulture Science, University of Tehran, Iran*
[5]*Department of Plant Breeding and Biotechnology, College of Agriculture, University of Tabriz, Tabriz, Iran*
[6]*Department of Agronomy and Plant Breeding, College of Agriculture, University of Ilam, Ilam, Iran*

ABSTRACT

Mariana (Prunus mariana) is one of the most important Root stocks of plum that is propagated by tissue culture. The problem of using explants of these Root stocks is contamination with fungus and bacteria and preparation of sterile explants is difficult. In this research, effects of different sterilizer compounds such as Sodium hypochlorite and 70% Ethanol in different concentrations and periods on contamination control of Mariana Root stocks were investigated. Bud establishing were done in MS medium. After four weeks percentages of budding, emerging and contamination were measured for different treatments. Results showed that using of Antibiotics in sterilizing process of explants with 10% Sodium hypochlorite, enhanced contamination control. Also the use of Cefotaxime Antibiotic (400 mg/l) in comparison with combination of Carbenicillin and Streptomycin antibiotics (each 200mg/l) is more effective on contamination control.

Key words: Mariana, Sodium hypochlorite, Cefotaxime, Carbenicillin, Streptomycin

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INTRODUCTION

Plums and prunes are systematically belonging to *Prunus* genus and Rosaceae family. In terms of taxonomic among the stone fruits, plums have the most diversity and are more compatible to the diverse climatic and soil condition [1]. Plums are native to west and central regions of China and later are brought to the central Asia, Iran, Armenia and Syria and have been cultivated. Plum has a favorable position in fruit industry economy. Development and growth rate, nutritional stress resistance in apricot trees are affected by physical and chemical conditions of soil of planted area.

Propagation of Mariana, typically due to genetic segregation and seedling weakness, is not cost effective and has many problems [2]. Despite to the great importance of stocks in development of fruit trees cultivation, due to presence of several problems in vitro culture of woody plants in comparison with herbaceous plants, there are a few reports about successful In vitro propagation of stone fruits. Now to overcome to the problems of conventional reproduction and to produce virus free plants, utilizing In vitro propagation in this rootstocks has became more important. Generally, terminal and lateral bud explants are used to reproduction of this rootstock [1]. Fungal and bacterial contaminations is a major problem in tissue culture and most of the contaminations of plant culture medium is made up of Escherichia coli, Pseudomonas, Erwinia and Bacillus bacteria [3]. In the cases that the purpose of tissue culture is commercially amplification of plant, bacterial and fungal contamination can cause irreparable damages. For surface disinfection typically 70% Alcohol, Sodium hypochlorite, Calcium hypochlorite, and Mercuric chloride is used. Also, Antibiotics and Silver nanoparticles are effective for the control of internal and external infections [4]. Internal infections usually appear several weeks after cultivation; these infections may be invisible, but affect the growth, division and emergency of the explants [5]. The aim of this study is to compare effects of Sodium hypochlorite and Cefotaxime, Carbenicillin and Streptomycin on control of contamination percentage of Mariana explants and to introduce the best concentration for increasing stability.

MATERIALS AND METHODS

In this research the Mariana plants were obtained from Parsaabad-E- Moghan Culture and Industry Center, and after labeling were transferred to Tissue Culture Laboratory of IRAN's North West and West Biotechnology Institute.

Different disinfecting components including Sodium hypochlorite and 70% Alcohol were used for disinfection of explants, in three steps with separate experiments. First, for investing the effects of different concentrations of Sodium hypochlorite on contamination rate of Mariana, experiment were performed in completely randomized experimental design with three replications and each replication include 20 explants. At this stage, disinfection treatments were considered as the first factor (Table 1). At the next stage of the experiment, the effects of different amounts of Antibiotics including: Carbenicillin, Tetracycline and Cefotaxime on explants contamination control against treatment of 70% Alcohol for one minute and 3% Sodium hypochlorite for 20 minutes was investigated using completely randomized experimental design with three replications and each replication include 20 explants. In the above stages, explants, after washing under running water and applying disinfection treatments were cultured on MS medium with additive 100mg/l of, Fe- EDTA, 20 mg/l of casein and 30 g/l of sucrose in 16 hours light condition at 25˚C. In the all stages, explants subculture were done after three weeks. At the all experiments four weeks after applying disinfection treatments, contamination rate were measured.

RESULTS AND DISCUSSION

In cases that the purpose of tissue culture is commercially amplification of plants, contamination control and preparing sterile explants is very important. Using disinfectant components is one of main ways to control of fungal and bacterial contaminations in the In vitro culture.

Results obtained from variance analysis of traits according to completely randomized experimental design showed that there is a significant difference between most traits (Table 1). Among traits the lowest coefficient of variation was belonged to the number of healthy plants

Table 1- Analysis of variance for measured traits

		Mean squares		
S.O.V	df	Browning of explants	Sprouting of explants	Contaminated plant
Treatment	3	66.972 **	38.00 **	56.750 **
Error	8	1.15	2.333	1.8334
CV (%)	-	11.48	7.94	12.13

***: Significant at %1 probability level*

(7.94), indicating that these traits were less affected by the environment. The number of infected plants with the highest coefficient of variation (12.13) was the most affected by the environment. Sodium hypochlorite as a general disinfectant is just capable to limited control of these contaminations [6]. Bacterial contaminations that have internal

origin and are within the tissue of explants commonly are not wiped out using surface disinfectants. In some cases, antibiotics may be useful for control of contamination of explants and reduce the percentage of infection. The results of this study showed that the use of antibiotics in explants disinfection process using 10% Sodium hypochlorite improved contamination control (Table2). Using Cefotaxime antibiotic (400 mg/l) compared with the combination of Carbenicillin and Streptomycin antibiotics (each 200 mg/l) was more effective to control of contamination (Table3), so that the separate use of this antibiotic in comparison with combined using of the two antibiotics has better condition in terms of contamination and emerging percentages [7]. Also emerged explants in medium containing Carbenicillin and Streptomycin, after subculture in the medium without Antibiotics, produced short foliage and rooting of them were initiate after longer time [8]. Bacterial infections are one of major problems in plant tissue culture, and generally control of such contamination especially internal types are difficult and they appear on the medium several weeks after cultivation [9]. Antibiotics are used in the disinfection process, before or after surface disinfection to control of bacterial contamination [10].

Table 2 - Comparison of effect of different levels of sodium hypochlorite on contamination controlling using Duncan's test at 1% level

Treatment levels	Browning of explants	Sprouting of explants	Contaminated plant
5	2.0 c	6.667 b	11.667 a
10	2.33 c	14.00 a	3.66 b
15	8.334 b	7.00 b	4.67 b
20	11.67 a	7.00 b	1.66 b

Table 3- Effect of Cefotaxime, Carbenicillin and streptomycin on percentages of contamination, Sprouting and Browning of explants of Rootstocks Mariana

Disinfectant treatments	Type and concentration of Antibiotic on the medium	Percent of Browning of explants	Percent of Sprouting of explants	Percent of contaminated plant
Hypochlorite 10%	without of Antibiotics	7 b	15 c	78 a
Hypochlorite 10%	Cefotaxime (400mg/l)	12 a	58.00 a	30 c
Hypochlorite 10%	Carbenicillin and streptomycin (400mg/l)	10 a	30 b	60 b

Different letters indicates significant differences
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