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DETERMINATION OF THE EFFECT OF DIFFERENT STERILIZATION PROTOCOLS FOR IN VITRO SHOOT TIP CULTURE IN SOME WILD PEAR (P. ELAEAGRIFOLIA) GENOTYPES

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Abstract
Wild pear(Pyrus elaeagrifolia) is one of the rootstocks used in pear cultivation. It is one of our most important genetic resources, especially because it is resistant to drought conditions and iron chlorosis. Besides its positive features, its use as a rootstock also has some disadvantages. In vitro propagation methods have gained importance in clonal propagation, especially due to the difficulties encountered in germination from seeds and problems in rooting with cuttings. With this method, mass production can be achieved in a short time. However, as in other species, contamination is the biggest obstacle in in vitro culture in pear. There is no valid protocol yet for surface sterilization processes for micropropagation of wild pear. New studies are needed for this purpose. The aim of this study was to determine the sterilization conditions suitable for the shoot tip technique to be used in in vitro propagation of some pear species. In the study, 4 different sterilization methods were applied to shoot tip explants of the wild pear genotype obtained from 6 different regions, and bacterial and fungal infection and plant development were examined in the explants. According to the results of present study, the contamination rate varied according to genotype and sterilization methods. The obtained results show that determining different protocols according to genotypes in in vitro micropropagation in important species such as wild pear (Ahlat) may affect the success of micropropagation.

Keywords: Wild pear, in vitro, sterilisation

1. INTRODUCTION

Pyrus elaeagrifolia is a wild pear species that grows widely in Anatolia. It is reported that the wild pear genus was cultivated approximately 3000 years ago and botanists have identified more than 24 species of wild pear to date. Two species (*P. elaeagnifolia*, *P. kotschyanus*) grow in Turkey. It is generally known by its common name: Ahlat. In some regions, it also has local names such as jackal pear and hyssop. Ahlat is a type of tree found almost everywhere in Anatolia. It is a species resistant to drought and air pollution. It is commonly found in arid places, forest clearings, anthropogenic steppes, especially in fields opened from the forest, where it is left for its fruit and shade, together with various hawthorn (*Crataegus*) species.

Wild pears gain importance because their fruits constitute a food source for humans and animals, are used in alternative and contemporary medicine, and are used in erosion control studies due to their widespread root systems. In addition, their resistance to diseases and pests, their ability to create aesthetic values in landscaping works due to their different crown forms, and their high

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ability to adapt to different climate and soil characteristics further increase the importance of these species (Gültekin et al., 2006). Wild pear is one of the young rootstocks used in pear cultivation. It is one of the most important genetic resources, especially because it is resistant to drought conditions and iron chlorosis. Besides its positive features, its use as a rootstock also has some disadvantages. In vitro propagation methods have gained importance in clonal propagation, especially due to the difficulties encountered in germination from seeds and problems in rooting with cuttings. With this method, mass production can be achieved in a short time. As is the case with the majority of fruit trees and nut species, the reproduction of uniform copies of an original *P. elaeagrifolia* parent plant is feasible through the utilisation of cuttings, layering, and micropropagation techniques. Among these techniques, only propagation by cutting has been the subject of study in this species. Two types of *P. elaeagrifolia* have been successfully propagated by softwood cuttings, with rooting percentages determined as 11.4 and 43.8% (Dumanoglu et al., 1999). Micropropagation enables fruit breeders to rapidly multiply a new rootstock in a relatively short period of time (Webster, 1995; Hartmann et al., 1997). The multiplication of axillary buds is a widely used method of mass propagation of plants that are genotypically and phenotypically identical to the original plant from which they were produced (Evans, 1990). The results of several studies on the micropropagation of various Populus species have been published, including *P. communis* (Iglesias et al., 2004), *P. calleryana* (Berardi et al., 1993), *P. calleryana* and *P. betulaefolia* (Yeo and Reed, 1995), and *P. syriaca* (Shibli et al., 1997; Chevreau and Bell, 2005). Nevertheless, there is a paucity of research on the micropropagation of *P. elaeagrifolia* (Thakur et al., 2008; Dumanoğlu et al., 2014; Aygun & Dumanoglu, 2015). There are many problems that affect the success of in vitro propagation of plants. One of these is the contamination seen in the starting material. As in other species, different in vitro sterilization methods have been applied by different researchers in wild pear(*Pyrus elaeagrifolia*) and different results have been obtained(Thakur et al., 2008; Dumanoğlu et al., 2014; Aygun, and Dumanoglu, 2015). The reasons for this difference may be the genetic structure of the material, the time of explant take, and the type of explant. There is no valid protocol yet for surface sterilization processes for micropropagation of Ahlat genotypes with different genetic structures. New studies are needed for this purpose.
The aim of present study was to determine the sterilization conditions suitable for the shoot tip technique to be used in the in vitro propagation of some wild pear species.

2. MATERIALS AND METHODS

6 different wild pear genotypes were used as plant material. Materials were collected from Kayseri, Nevşehir, Denizli and Uşak provinces in Turkey(Figure 1). Shoot tips were collected from fresh shoots during the spring development period and brought to laboratory conditions.

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Figure 1. Photos of the wild pear genotypes and shoots used in the study

For establishment media, MS (Murashige and Skoog, 1962) supplemented with 3% sucrose, 100mg/l myoinositol, %0,25 active charcol, 0,7% plant agar, 9.0 µM BA and 0.5 µM indole-3-acetic acid were used. For shoot proliferation, same media without indole-3-butyric acid (IBA) were used. For rooting, the medium containing half strength MS (Murashige and Skoog, 1962) medium supplemented with 5 µM indole-3-butyric acid (IBA) were used(Aygun and Dumanoglu, 2015). The pH of the media was adjusted to 5.7 before adding agar and autoclaving, and then these media were sterilized in the autoclave at 121 ºC for 20 minutes. After the autoclaving process, the sterile nutrient medium and culture containers were taken into the sterile cabinet and 10 ml of nutrient medium was distributed to each culture container.
Four different surface sterilization aprotocol were used for shoot tips of wild pear genotypes. And 50 explants were used for each protocols and total explant number were 200 for each genotypes.
Shoot tips were washed under running tap water for 5 minutes for all protocols.
Protocol 1: The explants were kept in 0.1% tween 20 with 3% sodium hypo chloride for 15 minutes (Aygun, and Dumanoglu, 2015).
Protocol 2: The explants were kept in 1% sodium hypo chloride for 20 minutes (Dumanoğlu et al., 2014).
Protocol 3; The explants were kept in 30% commercial sodium hypo chloride for 15 minutes.
Protocol 4: First, it was washed and rinsed with Tween 20 and then kept in 0.1% mercuric chloride for 4 minutes (Thakur et al., 2008).
Then, in each method, the shoot tips were rinsed three times with sterile pure water for all protocols. Microbial contamination ratio(%) shoot proliferation ratio(%) and rooted plant number(%), acclimatizated plants number (%) were determined for each genotypes and each protocol.

3. RESULTS AND DISCUSSIONS

6 different wild pear genotypes collected from different regions of Turkey were cultured under in vitro conditions with 4 different sterilization methods in the present study. As a result of all these studies, a serious variation in both proliferation and microbial contamination was observed between different genotypes.
In the present study, while some genotypes cause a lot of fungi/bacteria, some genotypes have a very low infection rate. the microbial contamination rate varied between 0-82%, the highest

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contamination was determined in the protocol P2 applied to genotype no. 1. In the protocol P4, 4% contamination was detected only in genotype 1, while no contamination was observed in other genotypes in the protocol P4. Shoot proliferation ratio (%) was obtained as 0% in the protocols P4 applied to all genotypes and in the protocol P3 applied to genotype 3. While rooting was obtained only in genotypes 4 and 5, acclimation was obtained from genotype 4 (2 plants) and genotype 5 (1 plant). Although protocol P4 was successful in terms of contamination, it prevented shoot development. The contamination rate was found to be high in protocols P1 and P2. However, the P3 (explants were kept in 30% commercial sodium hypo chloride for 15 minutes) protocol was determined to be the most successful method as it reduced the contamination rate although it reduced shoot development(Table 1; Figure 1.).

Table 1. Microbial contamination explant number, without microbial contamination explant number, microbial contamination ratio(%), shoot proliferation explant number, shoot proliferation (%) and rooted plant number(%) of Pyrus elaeagrifolia genotypes

Genotype number	Selected Province	Protocol code	Explant number	Microbial Contamination Explant number	Without Microbial Contamination Explant number	Microbial Contamination ratio(%)	Shoot proliferation explant number	Shoot proliferation (%)	Rooted plant number(%)	Acclimatizated plants number (%)
1	Kayseri	P1	50	35	15	70	5	7.1	0	0
		P2	50	41	9	82	3	3.7		
		P3	50	10	40	20	3	15.0		
		P4	50	2	48	4	0	0.0		
2	Kayseri	P1	50	30	20	60	6	10.0	0	0
		P2	50	33	17	66	5	7.6		
		P3	50	7	43	14	2	14.3		
		P4	50	0	50	0	0	0.0		
3	Nevşehir	P1	50	6	44	12	1	8.3	0	0
		P2	50	8	42	16	1	6.3		
		P3	50	1	49	2	0	0.0		
		P4	50	0	50	0	0	0.0		
4	Nevşehir	P1	50	10	40	20	6	30.0	3.3	2
		P2	50	18	32	36	3	8.3		
		P3	50	3	47	6	1	16.7		
		P4	50	0	50	0	0	0.0		
5	Denizli	P1	50	13	37	26	4	15.4	3.3	1
		P2	50	14	36	28	4	14.3		
		P3	50	1	49	2	1	50.0		
		P4	50	0	50	0	1	0.0		
6	Uşak	P1	50	15	35	30	5	16.7	0	0
		P2	50	23	27	46	5	10.9		
		P3	50	1	49	2	2	100.0		
		P4	50	0	50	0	0	0.0		

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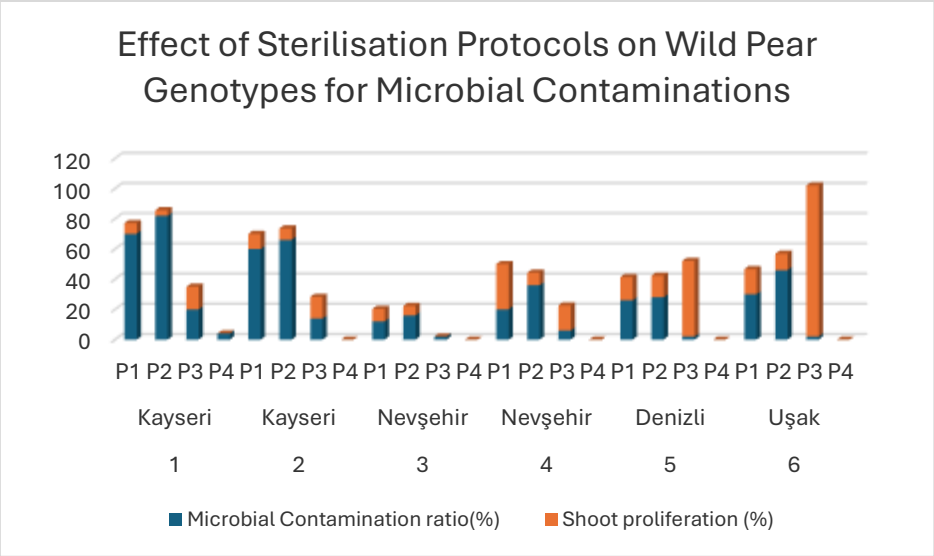

Figure 1. Effect of surface sterilisation protocols on Wild Pear genotypes for microbial Contaminants

Plant tissue culture methods are a valuable tool for obtaining disease-free and consistent planting materials, which can be made available for use for over 50 years (Eziashi et al., 2014). It is therefore evident that the method is of crucial importance for the micropropagation of Pyrus elaeagrifolia. A variety of contaminants have been identified in plant tissue culture, including fungi, bacteria, yeasts, viruses, and micro-arthropods such as mites and thrips (Cobrado and Fernandez, 2016). Microbial contamination can result in significant economic losses due to the expenditure of time, labour, and materials (Abass, 2013; Altan et al., 2010). Meanwhile, contamination is caused by phyllospheric, rhizospheric, and endophytic microorganisms that reside on, in, and inside the plants (Cobrado and Fernandez, 2016; El-Banna et al., 2021). In the absence of effective decontamination measures, contamination of explants can lead to a reduction in regenerative capacity, diminished callus growth, and the inhibition of adventitious shoot development (El-Banna et al. 2021; Safwat et al. 2015). Furthermore, the presence of microbial contaminants has been linked to an increase in plant mortality, variation in growth (reduced shoot proliferation and rooting), tissue necrosis, and even explant death (Ray and Ali, 2017). It is not possible for surface disinfection to remove internal bacterial contamination, whether intra- or intercellular. It should be noted that these bacteria are not always pathogenic or detrimental to plants in a natural environment. However, they have been observed to cause significant contamination during the in vitro propagation of plants (Permadi et al., 2023). Fungal contaminants represent a significant obstacle to the successful in vitro micropropagation of plants. These contaminants present a significant challenge at each stage of the in vitro plant culture process. Their formation and expansion occur more rapidly than the growth of the plant culture, resulting in the expenditure of time, resources, and, ultimately, a considerable economic loss (Mng'omba et al. 2012; Abass, 2013). The study revealed significant differences in Ahlat genotypes collected from various provinces and in vitro surface sterilisation methods employed. While the chemicals employed in the sterilisation process and the duration of their application are among the factors influencing the efficacy of in vitro sterilisation in micropropagation studies, the impact of genotype is also a crucial

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consideration. The structure of the explants, the manner of their development, the presence of natural hairs on the explant, and the natural pollination and waxy layer all affect the success of the sterilisation process. One of the issues identified in the research conducted on walnut is the low reproductive rate. It was hypothesised that the lengthy cultivation period and the presence of latent contaminants resulting from the slow growth of regenerated shoots were the underlying causes (Revilla et al., 1989). There are limited studies on surface sterilization applications in Ahlat in vitro micropropagation studies and there are no studies comparing different sterilization methods (Thakur et al. 2008; Dumanoglu et al., 2014; Aygün and Dumanoglu., 2015). In this study, it was observed that different strerilization methods affect the success of surface sterilization in *Pyrus elaeagrifolia* genotypes as in other species. It is thought that these differences are primarily due to the explant structure of the genotypes and then the chemicals and application time used in sterilization.

4. CONCLUSIONS

In this study, 4 different sterilization methods were applied to 6 different wild pear genotypes collected from different parts of Turkey and the contamination rate, shoot development rate, rooting and acclimation rates of the explants were determined. The present results show that contamination and plant development varied according to the genotypes and the applied protocols. The obtained results show that determining different protocols according to genotypes in in vitro micropropagation in important species such as wild pear(Ahlat) may affect the success of micropropagation.

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