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Surface Sterilization Optimization in Seeds of Şalak Apricot Variety (*Prunus armeniaca* L. cv. Şalak)

Yasemin KEMEÇ HÜRKAN[1*], Cüneyt AKI[2]

ABSTRACT: This research was carried out with the aim of improving the surface sterilization optimization technique in seeds which *in vivo* and *in vitro* growth of Şalak apricot cultivar. For seed sterilization, the effects of 0%, 10%, 15%, 20% NaOCl and 10, 15, 20 minutes exposure times were investigated. Seeds were kept in 70% ethanol for 40 seconds for pre-sterilization. The results of the study were evaluated as the rates of germinated seed (%) and contaminated seed (%). It was concluded that 20% NaOCl+15 minutes application for seed sterilization would be sufficient for surface sterilization with 12% contamination.

Keywords: Apricot, *Prunus armeniaca* L., surface sterilization, Şalak, NaOCl

[1] Yasemin KEMEÇ HÜRKAN (**Orcid ID:** 0000-0003-4089-2683), Çanakkale Onsekiz Mart Üniversitesi, Lisansüstü Eğitim Enstitüsü, Çanakkale, Türkiye

[2] Cüneyt AKI (**Orcid ID:** 0000-0002-7486-2282), Çanakkale Onsekiz Mart Üniversitesi, Fen Edebiyat Fakültesi, Biyoloji Bölümü, Çanakkale, Türkiye

***Sorumlu Yazar/*Corresponding Author*:** Yasemin KEMEÇ HÜRKAN, e-mail: kemecyasemin@gmail.com

Bu çalışma Yasemin KEMEÇ HÜRKAN'ın Doktora tezinden üretilmiştir.

INTRODUCTION

Turkey is a very rich country in terms of climate and soil properties as well as underground and surface resources. Turkey has an important place among other world countries in terms of fruit variety, species and production amount. Anatolia is the gene source of *Prunus cerasus* L., *Prunus avium* L., *Pyrus* sp. Linnaeus, *Malus domestica* (Suckow) Borkh, *Cornus mas* L., *Punica granatum* L., *Juglans* sp. L., *Castanea* sp. Mill., *Prunus dulcis* Mill., *Corylus* sp. L., *Pistacia vera* L., *Olea europaea* L., *Vitis* sp. L. and *Ficus carica* L. Other fruit varieties used for various purposes are *Rubus* sp. L., *Prunus mahaleb* L., *Pistacia khinjuk* Stocks, *Prunus laurocerasus* L., *Rosa canina* L., *Crataegus monogyna* Jacq., *Celtis* sp. L., *Elaeagnus* sp. L., *Ceratonia siliqua* L. and many more fruit trees that grow naturally (Asma, 2011).

Another fruit that has been produced and traded in the fertile soils of Anatolia for 2500 years is apricot. Apricot (*Prunus armeniaca* L.) is in the group of stone fruits belonging to the Rosaceae family. Apricot is divided into 6–8 ecological groups and 13 regional subgroups due to its propagation by seed and growing in a wide range of different ecological conditions. The gene source of apricot involves a wide area from Central Asia to Northern China (Asma, 2011; Layne et al., 1996; Ledbetter, 2008). Apricot is an important fruit for human health and is rich in sugar, phosphorus, iron, potassium, calcium, dietary fiber, A (β-carotene) and vitamin C. Apricot is also a very useful fruit in terms of protecting against infection and cancer by increasing body resistance, eye health, functioning of endocrine glands, epithelial tissue, teeth and bone development (Açkurt, 1999; Otlu et al., 2008; Yücecan, 1994).

Turkey has 58 apricot cultivars, 28 of which are registered apricot cultivars (Table 1). Şalak is an edible apricot variety grown in Iğdır and Kağızman regions. Its fruits are oblong (long) and very large, with an average fruit weight of 50–65 g. The fruits are sweet, the pericarp and pulp color are yellow, the shape is symmetrical and the abdominal line is very prominent. The weight of the seeds is 2.1–2.6 g, the shape is long and sweet. Its total acidity is 0.30–0.50%, pH is 4.4–4.8 and the amount of water-soluble dry matter is 17–20%. It ripens in the last week of June under the climatic conditions of Iğdır and Malatya (Asma, 2011; Aydoğdu, 2016).

Table 1. Local and registered apricot varieties (Asma, 2011; Anonim, 2022)

Local and Registered Apricot Varieties		
Hacıhaliloğlu*	Çataloğlu*	Şekerpare*
Hasanbey*	Çöloğlu*	Dilbay
Kabaaşı*	Alyanak*	Mahmudun Eriği*
Soğancı*	Aprikoz (Şalak)*	Adilcevaz-5
Tokaloğlu-Erzincan	İmrahor*	Hacıkız
Tokaloğlu -Yalova	Kuru Kabuk*	İsmailağa
Tokaloğlu-Konya Ereğli	Şam*	İri Bitirgen*
Tokaloğlu-İzmir	Turfanda İzmir*	Karacabey*
Çiğili İzmir*	Çekirge 52	Şahinbey
Sakıt-1-2*-3-4-5-6-7	Ağerik	Mektep*
Turfanda Eskimalatya*	Hırmanlı	Proyma*
Ethembey*	Geç ve Güz Aprikoz	Levent*
Özal	Kamelya	Kadıoğlu
Ziraat Okulu	Roxana*	Casna Drenova*
Kayısı Eriği	İnciaz Eriği	Alkaya*
Alatayıldızı	Stark Early Orange*	Çağrıbey
Çağataybey	Ordubat*	Dr. Kaşka

* Registered apricot varieties

In Turkey, apricot cultivation is carried out of Malatya, Elazığ, Erzincan, Sivas, Kahramanmaraş, Mut, Isparta, Hatay ve Antalya (Mediterranean Region), Iğdır-Kağızman, Afyon, Manisa ve İzmir

(Aegean Region), Kayseri, Konya, Ankara, Nevşehir, Niğde, Yozgat, Karaman ve Aksaray (Central Anatolia Region), Çanakkale, Balıkesir, Tekirdağ ve Edirne (Marmara Region) (Asma, 2011; Aydoğdu, 2016). Malatya ranks first in apricot production area with 64.2%. Malatya is followed by Elazığ (7.6%), Kahramanmaraş (6.8%), Mersin (6.2%), Iğdır (2.7%) and Isparta (1.9%). In the 2019 production period, a total of 864 thousand tons of apricots were produced in Turkey, including wild apricot. Malatya ranks first with a production of 392 thousand tons. It constitutes 46.3% of Turkey's production. Mersin (16.6%), Kahramanmaraş (7.7%), Elazığ (6.6%), Iğdır (4.7%) and Hatay (3.7%) follow Malatya, respectively. While Malatya ranks first in dried apricot production, other provinces are Elazığ, Sivas, Kahramanmaraş and Mersin, respectively (TÜİK, 2022).

The first step to initiate healthy and pathogen-free cultures in both *in vitro* and *in vivo* studies; is the optimization of surface sterilization technique. Since the explant to be studied is obtained from the apricot plant grown in garden conditions, various microorganisms can easily contaminate cultures both internally and externally. There are many studies involving different sterilization methods in which different concentrations and retention times of NaOCl are applied in apricot and other stone fruit species. Yıldırım (2006), conducted sterilization optimization and organogenesis studies by using seeds, nodal buds and shoot tips of Hacıhaliloğlu apricot cultivar. For the sterilization study, the seeds in 5% NaOCl (sodium hypochlorite) for 15 mins, the nodal buds in 5% NaOCl for 10 mins and the shoot tips in 10% NaOCl for 15 mins determined that it would be appropriate to keep. Wang et al. (2011), studied on adventitious shoot regeneration from hypocotyl slices of mature seeds of "Canino", "Dorada" and "Moniqui" apricot cultivars. For the sterilization process, the seed whose endocarp was broken was kept in 100 ml sterile distilled water containing 1% NaOCl and 20 µL Tween-20 for 20 mins. After rinsing four times with sterile distilled water, it was kept in sterile distilled water at 4 ºC overnight. Wang et al. (2013), used cotyledons of mature seeds obtained from four apricot cultivars (Canino, Dorada, Real Fino, Moniquí) and one rootstock ["ansu Maxim" (*P. armeniaca* L. var. "ansu Maxim")] as explants in their study. For the sterilization process, the seed whose endocarp was broken was kept in 100 mL sterile distilled water containing 1% NaOCl and 20 µL Tween-20 for 20 mins. After rinsing four times with sterile distilled water, it was kept in sterile distilled water at 4 ºC overnight. Yıldırım (2012), used 15 different apricot varieties in his study. For the sterilization process, the seeds were pre-sterilized with 70% alcohol for 45 secs and rinsed with sterile distilled water. Then, the seeds were surface sterilized in 5% NaOCl (Sodium Hypochlorite 53%-Axion) for 15 mins and then rinsed with sterile distilled water 3 times for 5 mins. For easier peeling of the seed coat and easier separation of the embryos, the seeds were kept in sterile distilled water for 1 hour. Yıldırım et al. (2011), conducted a surface sterilization optimization study on explants such as single node, shoot tip and seed of Hacıhaliloğlu apricot cultivar. In this study, the effects of different concentrations of sodium hypochlorite (0.5%, 10%, 15% and 20%) and different rinsed times (5, 10, 15, 20 and 30 minutes) (at the most effective concentration of NaOCl) for sterilization were investigated. As a result, it was determined that waiting in 10% NaOCl for 15 mins for sterilization of shoot tips, 5% NaOCl for 10 mins for sterilization of single node explants, and 15 mins in 5% NaOCl for sterilization of seeds was found to be suitable for optimization of surface sterilization. Mante et al. (1989), investigated plant regeneration from the mature seed of the *Prunus domestica* L. and *P. cerasus* L. plants and from the proximal part of the cotyledons (embryonic axis removed) of the immature seed of the *P. persica* L. plant. For the sterilization process, the endocarp of the seeds was broken, the cotyledons were separated and the embryonic axis was removed. Seeds were washed under running tap water for 5 mins. Disinfected with 0.5% sodium hypochlorite (a few drops of 1% Triton x 100 with 10%

commercial bleach) for 12–15 mins. After disinfection, it was rinsed three times with sterile distilled water. The disinfected seeds were soaked in sterile distilled water overnight.

In this research, the optimization of seed surface sterilization of apricot seeds, which is highly produced in Turkey and ranks first in the world in terms of production, has been examined.

MATERIALS AND METHODS

Materials

In this research, mature seeds of Şalak apricot variety of *Prunus armeniaca* L. plant belonging to Rosaceae (Rosaceae) family (tree age 10–12) were used (Figure 1). All of the materials used in the study were obtained from Iğdır University Agricultural Application and Research Center. The collected apricot fruits were washed after being separated from their fleshy parts and dried in a cool and shaded place.

Figure 1. Tree, fruit and seed form of the material used, respectively.

Surface sterilization of seeds

Before surface sterilization, the endocarp of apricot seeds was broken with a hammer. After the seeds were washed under running tap water for 3–5 mins, they were pre-sterilized in 70% ethanol for 40 secs. Surface sterilization was provided in sodium hypochlorite (NaOCl) solution at different times and concentrations (Table 2). After sterilization, it was rinsed 3 times with sterile distilled water. In order to facilitate the peeling of the testa on the seed and to reduce the activity of abscisic acid, the seeds were kept in sterile distilled water at 4ºC ± 1 for 24 hours.

Table 2. Surface sterilization of apricot seeds

Sterilization Number	Minute (min)	NaClO (%)
1	10	10%
2	15	10%
3	20	10%
4	10	15%
5	15	15%
6	20	15%
7	10	20%
8	15	20%
9	20	20%

Statistical analysis

All of the data obtained from this study were evaluated by making ANOVA in the XLSTAT 2021 statistical package program according to the randomized plots trial design. After the statistically significant transactions were determined, the differences between the averages were determined with the Duncan test at the p=0.05 level. Obtained data are given in tables as mean ± standard deviation. The following significance levels were used in the analyses:

p>0.05 = not significant

p<0.05 = significant

$p<0.01$ = very significant

$p<0.001$ = quite a lot significant

RESULTS AND DISCUSSION

The effect of 10%, 15% and 20% concentrations of NaOCl and different exposure times of 10, 15 and 20 mins on the surface sterilization of seeds of "Şalak" apricot variety were tested. Statistical data are given in Table 3. There is a statistically significant difference ($p<0,05$) between different concentrations of NaOCl and different exposure times used in the study. In the study, it was concluded that 20% NaOCl + 15 mins application would be sufficient for surface sterilization with 12% contamination. Although the percentage of contamination was found to lower with 10% in 20% NaOCl + 20 mins application, seed germination was lower with 66% compared to 20% NaOCl + 15 mins application (88%).

Table 3. The effect of different concentrations and exposure of NaOCl on the surface sterilization of seeds

Sterilization Number	Contamination (%)	Germination (%)
Control	88.90±0.335^{a}	15.56±0.391^{f}
1	86.00±0.229^{a}	30.00±0.250^{e}
2	76.00±0.374^{b}	46.00±0.502^{d}
3	68.00±0.245^{b}	50.00±0.512^{d}
4	74.00±0.391^{b}	48.00±0.320^{d}
5	66.00±0.229^{b}	52.00±0.600^{cd}
6	52.00±0.332^{c}	54.00±0.450^{cd}
7	46.00±0.502^{c}	62.00±0.350^{bc}
8	12.00±0.332^{d}	88.00±0.245^{a}
9	10.00±0.250^{d}	66.00±0.320^{b}
	***p*<0.0001**	***p*<0.0001**

Since apricot is a horticultural crop, explant parts such as shoots, nodal buds, embryos and seeds are suitable places where a number of bacterial and fungal microorganisms can live and shelter, both externally and internally. If working with a fruit tree such as apricot in *in vitro* or *in vivo* studies, and if the explant has to be taken directly from the tree, not from a plant grown under controlled conditions such as a laboratory, then the first priority should be to ensure the surface sterilization optimization of the explant to be studied. In this study, domestos sterilant containing ≤5 NaOCl was used. Some researchers have used commercial sterilants containing 10% and 53% (Axion) NaOCl (Mante et al., 1989; Yıldırım, 2006, 2012; Yıldırım et al., 2011). While Yıldırım (2006, 2011, 2012), determined that 5% NaOCl + 15 mins application in apricot seeds gave better results for sterilization, Mante et al. (1989) found that 0.5% + 12–15 mins application gave better results for sterilization. Wang (2011, 2013), stated that the application of 1% NaOCl and 20 µl of Tween-20 for 20 mins would be sufficient for sterilization of apricot seeds. In this study, we decided that 20% NaOCl + 15 mins waiting time is sufficient for sterilization of apricot seeds.

CONCLUSION

In general, the sustainability of *in vitro* and *in vivo* studies depends on the optimization of the surface sterilization of the material to be studied. Developing the most suitable surface sterilization techniques is a very important step, as the concentration and duration will differ depending on the plant species, cultivar and explant type to be studied. For example; It is known that different sterilant, pre-sterilization, different concentration and exposuring times are applied in surface sterilization studies of different explant (shoot tip, meristem, single node buds, embryo and seed, etc.) types of various apricot and stone fruit species. In some studies, it has been reported that the explant to be studied is pre-sterilized with detergent-based materials such as fungicide or Tween-20, and then the

normal sterilization process is continued. It is possible that there will be differences in sterilization practices due to factors such as the condition of the plant from which the explant will be taken, the type and variety of the plant, the season and period from which the material will be taken.

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Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

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