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In Vitro Embryo Culture of Apricot, *Prunus armeniaca* L. cv. Hacıhaliloğlu

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Abstract: An *in vitro* zygotic embryo germination protocol was developed for apricot (*Prunus armeniaca* L. "Hacıhaliloğlu"). A hundred percent of germination was obtained when isolated embryos were cultured *in vitro*, a result alike those reported in the literature with traditional dormancy breaking treatments. *In vitro* seedling development was optimal in a modified full-strength Murashige and Skoog (1962) [1] medium with Gamborg vitamins supplemented with 1 mgl^{-1} BA, 30 gl^{-1} sucrose, 6.3 gl^{-1} agar. An average of 2-4 shoots was produced from the initial seedlings after 14 days of culture. *In vitro* developed plantlets were successfully acclimatized and transferred to soil. *In vitro* embryo culture thus allows for the production of apricot seedlings that can be transferred to natural orchards.

Keywords: apricot, germination, Hacıhaliloğlu, zygotic embryo

Abbreviations: 6-BA - 6 benzyladenine; MS - Murashige and Skoog medium; PGR(s) - plant growth regulator(s); Kin – kinetin; IAA - indole-3-acetic acid; NAA – α –naphthalene acetic acid; 2,4-D – 2,4-Dichlorophenoxyacetic acid

Hacıhaliloğlu Kayısı Çeşidinin *İn Vitro* Embriyo Kültürü

Özet: Bu çalışmada; Hacıhaliloğlu kayısı çeşidi için *in vitro* koşullarda embriyo çimlendirme protokolü geliştirilmiştir. İzole edilen embriyoların *in vitro* şartlardaki çimlenme oranı %100 olarak bulunmuş olup; normal şartlarda kayısı tohumlarında bulunan dormansiyi ortadan kaldırmak amacıyla farklı çalışmalarda benzer uygulamaların da yapıldığı görülmüştür. *In vitro* koşullarda tam MS besi ortamı kullanılırken; bu ortam Gamborg vitaminleri, 1 mgl^{-1} BA, 30 grl^{-1} sukroz ve 6.3 grl^{-1} agar ile desteklenmiştir. Çalışma sonucu gelişen bitkiler başarılı bir şekilde aklimatize edildikten sonra toprağa aktarılmıştır. Böylece arazi koşullarındaki kullanım için fidan üretimi amacıyla da bu yöntemin kullanılabileceği sonucu ortaya çıkmıştır.

Anahtar Kelimeler: Çimlenme, Hacıhaliloğlu, kayısı, zigotik embriyo.

1. Introduction

Apricot (*Prunus armeniaca* L.) is mostly grown in the Mediterranean countries, Russia, USA, Iran and Pakistan. Total world production of fresh apricot is 2.62 million tons/years (2002-2005 years average) [3]. Turkey is the leading producing country both for fresh and dried apricot. Total fresh and dried apricot production of Turkey was (2002-2005 years average) 383 thousand metric tons, respectively, composing a 15-20% fresh and 65-80% dried apricot production of the world [2,3].

Apricots are grown throughout Turkey, except in the very humid region around the Black Sea and cold mountain area of Anatolia. The most important apricot growing region is the Eastern Anatolia. Malatya, a province in this region, is the most important apricot production center of the country [4]. Malatya produces 50% fresh and 90% dried apricot of the whole country [5]. The important apricot cultivars in the Malatya region are Hacıhaliloğlu, Kabaaşı, Hasanbey, Soğancı and Çataloğlu. Malatya is known as the biggest dried apricot export center as 80-85% world apricot export [4,3].

In the current horticultural practice, the propagation of apricot is achieved only from seed, budding or grafting. To propagate elite varieties grafting and budding methods must be employed, since difficulties in the rooting of the stem cuttings from mature fruit bearing trees prevent traditional propagation methods such as stem cuttings. Rootstocks of apricot are

produced from seeds. Propagation form seed is desirable because it is logistically simple. As a rootstock for apricot, the seedlings from *P. armeniaca* L. species are widely used throughout the world [6, 7]. In addition to wild apricot types, the seedlings from seeds of cultivated varieties might be used as a rootstocks for apricot [8]. One of the most desirable characteristic in the apricot seedling rootstock is that the seeds might show a high germination percentage. The dormancy seen in the temperate climate fruit types hinder germination of the seeds, therefore, in order to overcome the dormancy problem in seeds, stratification, mechanical scarification, a seed scarification, hydratization in the water, growth promoter and vitamin application are the most practiced processes [9]. *In vivo* germination percentages of some apricots mature seeds treated with different vitamins were reported by Ercisli et al. [8] and the highest mean germination percentage was observed in the cultivar of Şekerpare (55-69%) while the lowest mean germination percentage was observed in Hacıhaliloğlu (23.06%) cultivar.

Up to know little is known of the effect of *in vitro* culture factors on germination of zygotic embryos of *P. armeniaca* cv. Hacıhaliloğlu. Therefore, the aim of the present study was to develop an *in vitro* embryo culture protocol for apricot (*P. armeniaca* L. cv. Hacıhaliloğlu) and propose an alternative or complement to existing germination techniques. The reported information would also be useful in future breeding experiments with the variety.

2. Material Method

2.1. Plant Material

Mature seeds from open pollinated of *P. armeniaca* L cv. Hacıhaliloğlu trees were collected from Malatya Fruit Research Institute in Turkey. Seeds were stored in plastic bags at 4°C as the source of embryos for all experiments.

2.2. Seed germination

Fifty seeds were sown into pots under glasshouse conditions in substrate compost of soil and peat (1:1). The pots were covered with a plastic film to maintain high relative humidity. This experiment was repeated thrice. Observations were recorded two months after inoculation for glasshouse experiments.

2.3. Surface Sterilization

Mature kernels from fruits the outer pericarp and shells had been removed, were pre-sterilized by immersion in 70% ethanol for 45 sec followed by a rinse with sterile distilled water. These pre-sterilized kernels were then exposed to 5% (v/v) sodium hypochlorite (Axion-%53 NaOCl) solution for 15 min. The kernels were washed 3 times with sterile distilled water. The testas were removed and the zygotic embryos were then isolated with a sterile scalpel before being placed in the culture medium.

2.4.*In vitro* Germination

After disinfection, 18 whole seeds and 18 isolated zygotic embryos were cultured *in vitro* on a germination medium compost of Murashige and Skoog Medium (MS) [1], 30 gl^{-1} sucrose, 1 mgl^{-1} BA and 6.3 gl^{-1} agar (Merck). The pH of this medium was adjusted to 5.7 prior to autoclaving for 15 min at 121°C. To test the effects of plant growth regulators, the auxins NAA, IAA and 2,4–D and the cytokinins such as BA and Kin were studied each at 1 mgl^{-1}. Different carbohydrates (sucrose, glucose, fructose and maltose) and various concentrations of sucrose (1, 2, 3, 4, 5 and 6%) were tested in MS medium. The effect of light on embryo germination was also investigated by culturing the isolated embryos in complete darkness and continuous light. Light was provided by cool white fluorescent tubes located at 30-35 cm above the self, a photo flux density was 40 µmol $m^{-2}s^{-1}$. These experiments were repeated at twice (for a total of 18 replicates). Observations were recorded 14^{th} day after incubation for *in vitro* experiments. The dissection of the seeds and isolation of the embryos were done using a binocular microscope, forceps and a scalpel. The explants were placed in test tubes containing 10 or 50 ml of medium, depending on the type of culture tubes used, and the tubes were randomly placed on racks. The racks were randomly distributed in the growth room.

2.5. Acclimatization

In vitro developed plantlets were maintained for 3 weeks in an acclimatization module under high relative humidity, after which the humidity was gradually reduced to 50%. The plants were grown in greenhouse and observed periodically.

2.6. Statistical Analyses

A randomized complete block design was used for all experiment. Each treatment was replicated at least twice, and each replicate consisted of 18 zygotic embryos. Significance was determined by analyses of variance (ANOVA) and the least significant (P=0.05) differences among mean values were estimated using Duncan's multiple range test. Data presented in percentage were subjected the chi-square (χ^2) test.

3. Results

Only 50% of the seeds was germinated in glasshouse, even two months after sowing of the seeds (Table 1). However, 75% of the whole seeds were germinated *in vitro* on the germination medium 14 days after culturing, and 85% of the germination occurred when the isolated zygotic embryos were cultured *in vitro*.

Table 1. Greenhouse and *in vitro* germination of apricot.

Type of culture	Explant	Number of explant cultured	Germination* (%)
Greenhouse	Seed	150	50
In vitro	Seed (kernel)	54	75
In vitro	Isolated embryo	54	85
χ^2 **(df : 2)**		-	$P \leq 0.05$

*The percentage presented here is a compilation of data obtained in the three replicates of this experiment. Data were recorded on the 14th day of incubation for *in vitro* and 60th day for glasshouse experiments.

Morphological Development. In vitro zygotic embryo germination in apricot started after 3 days of culture. Root elongation occurred within 72 h after incubation and shoot elongation phase started 5-7 days after initiation and continued over the culture period (Fig 1.)**.** By day 5, a leaf shoot occurred and by day 7, the first leaf emerged through the leaf sheath. Leaf development was slower than that of the root, but within 14 days, a second or third leaf has emerged and associated adventitious roots were clearly discernable (Fig 2.) No embryos developed with healthy-looking plantlets in the NAA and 2,4-D supplemented media. In general, the mean shoot length or root length was delayed or inhibited by the auxin supplemented media and was induced callus from the radicle tips.

There was significant difference in the frequency of germinated seeds among the plant growth regulators applied, as presented in Table 2 ($P<0.05$). After 14 days of culture, the percentage of the zygotic embryos germinated was the highest (100%) in the cytokinin BA treatment among plant growth regulators tested. The frequency of germination rates was gradually decreased to 67%, 44%, 11% and 11%, respectively in Kin, IAA, NAA and 2,4-D treatments. BA supplemented treatment produced the highest mean shoot length (14.03 ± 1.34 mm) but the mean root length which as significantly lower than the Kin, IAA and the control treatments. The control group produced significantly highest mean root length.

Data given in Table 2 show that type of plant growth regulators does effect zygotic embryo germination. From the results, it would appear that the cytokinin BA was superior to other tested treatments in terms of its ability to germinate the zygotic embryos of apricot. It also appears that the tested growth regulators especially the auxins had an inhibiting effect on the healthy germination of zygotic embryos compared to the control treatment.

3.1. Effect of Plant Growth Regulators

Table 2. The effect of plant growth regulators on germination of zygotic embryos of apricot.

Plant	% of	Shoot length	Root

Growth Regulators (1 mgl^{-1})	germination embryos	± SE (mm)	length ± SE (mm)
Control	72	6.83 ± 1.13 bc	21.58 ± 2.93 a
BA	100	14.03 ± 1.34 a	8.31 ± 0.90 c
Kin	67	9.34 ± 2.51 b	14.61 ± 1.62 b
IAA	44	6.11 ± 1.50 bc	14.34 ± 2.60 b
NAA	11	2.93 ± 0.71 cd	2.97 ± 1.47 d
2,4 D	11	1.85 ± 0.10 d	1.57 ± 0.07 d
χ^2 (df: 5)	P ≤ 0.05	-	-
s.d: 5.45 (F:4.401)	-	P ≤ 0.05	-
s.d: 5.48 (F: 7.894)	-	-	P ≤ 0.05

Data were recorded on the 14th day of incubation represents on average 18 replicates per treatment. Means in a column followed by the same letters are not significantly different at P=0.05 according to Duncan multiple range test.

3.2. Effect of Carbohydrate Source

The influence of various carbohydrate sources on the germination of zygotic embryos is shown in Table 3. There were no significant differences in the frequencies of the germinated embryos among treatments (P>0.05). The germination frequency rate with control (sugar-free) and fructose (94%) after 14 days culture was lower than the percentage of embryos germinated on the other treatments.

Table 3. The effect of sugar on germination of zygotic embryos of apricot.

Treatments	% of Germ. embryos	Shoot Length (mm)	Root length (mm)
Control	94	3.00 ± 0.21 c	2.16 ± 0.28 c
Sucrose	100	14.03 ± 1.34 a	8.31 ± 0.90 ab
Glucose	100	15.94 ± 1.37 a	8.70 ± 0.65 a
Fructose	94	15.19 ± 1.07 a	7.53 ± 0.81 ab
Maltose	100	9.14 ± 0.75 b	7.18 ± 0.35 b
χ^2 (df: 4)	P > 0.05	-	-
s.d: 4.72 (F:20.086)	-	P ≤ 0.05	-
s.d: 4.71 (F: 10.676)			P ≤ 0.05

Data were recorded on the 14th day of incubation represents on average 18 replicates per treatment. Means in a column followed by the same letters are not significantly different at P=0.05 according to Duncan multiple range test.

In terms of mean shoot length, there was significant difference among the tested carbohydrates. After 14 days of culture, an average of 15.94 mm shoots was produced on glucose supplemented medium which were significantly higher than the control and maltose supplemented media. Similarly, the mean root length varied among the tested carbohydrates. The mean root length was significantly high on glucose supplemented medium. The lowest mean of root length was obtained the control group. Through these results, it may be suggested that no embryos developed with healthy-looking plantlets in the control treatment. However, the embryos cultured on carbohydrate supplemented medium, of all them were germinated and developed new plantlets.

From these results it may also be suggested that healthy embryo germination depends on the carbohydrate type and concentrations, because the carbohydrate types tested in the MS medium had a pronounced effect on shoot and root length.

3.3. Effect of Sucrose Concentrations

The effect of various concentrations of sucrose on zygotic embryo germination is presented in Table 4. There were significant differences in the frequencies of the germinated embryos among treatments. 14 days after culture, 83%, 72%, 100%, 94%, 72% and 72% of cultured embryos were germinated on the MS medium supplemented with 1, 2, 3, 4, 5 and 6% sucrose, respectively. According to quantitative evaluation of the germinated zygotic embryos, the longest shoot length (14.03 ± 1.34 mm) as developed on 3% sucrose and the longest root length (13.04 ± 2.26 mm) occurred on the 5% sucrose treatment. On the basis of these results, 3% sucrose may be selected as a primary carbohydrate source.

Table 4. The effect of sucrose concentrations on germination of zygotic embryos of apricot.

Treatments	% of Germ. embryos	Shoot length (mm)	Root length (mm)
1 %	83	6.27 ± 0.92 b	8.10 ± 1.40 b
2 %	72	6.58 ± 0.99 b	8.26 ± 1.40 b
3 %	100	14.03 ± 1.34 a	8.31 ± 0.90 b
4 %	94	7.11 ± 0.90 b	10.69 ± 1.13 ab
5 %	72	7.67 ± 0.83 b	13.04 ± 2.26 a
6 %	72	7.91 ± 0.88 b	11.24 ± 1.44 a
χ^2 (df: 5)	P ≤ 0.05	-	-
s.d: 5.71 (F:8.826)	-	P ≤ 0.05	-
s.d: 5.62 (F: 1.976)	-	-	P ≤ 0.05

Data were recorded on the 14th day of incubation represents on average 18 replicates per treatment. Means in a column followed by the same letters are not significantly different at P=0.05 according to Duncan multiple range test

3.4. Effect of Light on Embryo Germination

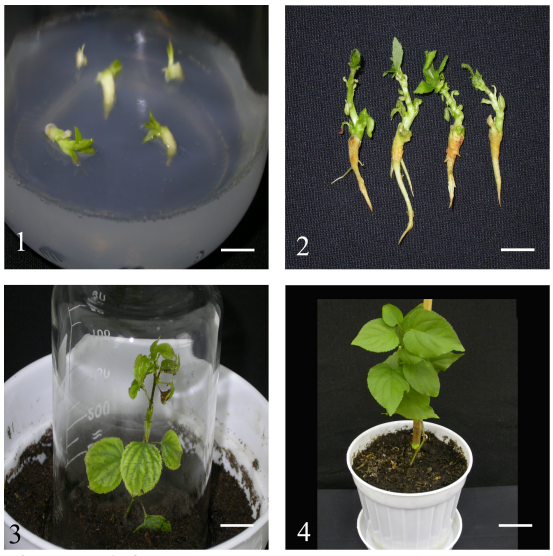
There was no significant difference in the frequencies of the germinated embryos among the treatments, as presented in Table 5. In terms of mean shoot length, there was significant difference between the treatments tested. After 14 days of culture the mean shoot length was significantly higher on complete darkness, but the leaves and shoot produced in complete darkness were tiny and unhealthy. However, the developing plantlets were then transferred to the standard incubation conditions for another 2 weeks, and then all of the plantlets became green.

Table 5. The effect of light on germination of zygotic embryos of apricot.

Treatments	% of Germ. embryos	Shoot Length (mm)	Root Length (mm)
Continued Light	100	14.03 ± 1.34 b	8.31 ± 0.90 a
Complete Darkness	100	18.40 ± 1.90 a	9.29 ± 0.70 a
χ^2 (df: 1)	P > 0.05	-	-
s.d: 1.34 (F:3.527)	-	P ≤ 0.05	-
s.d: 1.31 (F: 0.688)	-	-	P > 0.05

Data were recorded on the 14th day of incubation represents on average 18 replicates per treatment. Means in a column followed by the same letters are not significantly different at P=0.05 according to Duncan multiple range test.

All the plantlets acclimatized (Figure 3) were transferred to a mixture of soil and peat. Shoots grew normally and continued producing additional shoots (Figure 4).



Figures 1-4: Plantlet regeneration from mature zygotic embryos in apricot

Fig 1. Germinating zygotic embryos, seven days after culturing, bar: 10 mm.
Fig 2. Germinated zygotic embryos two weeks week after incubation, bar: 12, 5 mm.
Fig 3. Acclimatizing zygotic seedlings three weeks after incubation, bar: 18, 2 mm.
Fig 4. Regenerated plantlet established in a mixture of peat and soil, bar: 45 mm.

4. Discussion

In vitro seed germination has not been previously reported for the cultivar of "Hacıhaliloğlu". In our study, the highest germination rates in two weeks occurred on MS medium under continued light or a 16 h photoperiod at 25 ± 2 °C. Many of the previous glasshouse germination studies were published in conference proceedings [10,8]. These studies do not include complete data from germination through acclimatization. We examined complete data of the effects of plant growth regulators, carbohydrate type, different concentrations of sucrose, dark and light incubation on seed germination, and subsequent seedling development.

Several studies have shown that apricot seeds are dormant and that dormancy breaking treatments have to be performed to obtain high germination and that the dormancy being caused by the permeability of the seed coat and fruit pericarp rather than by the embryo [4,9]. Stratification, scarification, hydratization in water, growth promoter and vitamin application usually increase the germination percentage of the seeds [11, 12]. For "Hacıhaliloğlu", Esitken et al. [13] showed that seeds collected in the fall was dormant and obtained a germination percentage of 65% with a dormancy breaking treatment consisting of vitamin applications, and Uslu et al. [10] reported a maximum germination percentage of 71%. The present results show that a hundred percent of Hacıhaliloğlu zygotic embryos can germinate and grow when isolated and cultured on MS medium with Gamborg vitamins supplemented with 1 mgl^{-1} BA, 30 gl^{-1} sucrose and 6.3 gl^{-1} agar, and placed in a culture room at 25 °C ± 2 °C provided with a 16 h photoperiod through standard fluorescent lamps. Considering the results obtained, it can be concluded that *in vitro* conditions favored germination more than the glasshouse

conditions. In a commercial setting, a seed culture protocol that proceeds a higher quality, as well as large seedlings in a short period of time is available. *In vitro* embryo culture of Hacıhaliloğlu is thus an alternative, or complement to existing germination techniques. The present study not only enables a large number of aseptic seedlings to be produced in a short period of time but it also helps to speed up apricot breeding programmes.

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6. References

1. Asma, B.M., (2000). Apricot Growing. Evin Pres. Malatya. Turkey (In Turkish), 243s.
2. Asma, B. M., Ozturk, K. (2005). Analysis of morphological, pomological and yield characteristics of some apricot germplasm in Turkey. *Genetic Resources and Crop Evolution*, **52** : 305-313.
3. DIE, (2002). Agriculture Statistics. Prime Ministry Pres. N: 2885, Ankara, 592s.
4. Eriş, A., (1990). Bahçe Bitkileri Fizyolojisi. U.Ü.Z.F. Ders Notları No: 11, 152s.
5. Ercisli, S., Esitken, A., Guleryuz, M., (1999). The effect of vitamins on the seed germination of apricot. In: Proc. XI. Int. Symp. On Apricot Culture. **488** (2): 437-440.
6. Eşitken A., Güleryüz M. and Ercişli S. (1999). Embryo Culture of Hasanbey Apricot cv., XIth Int. Symp. on Apricot Culture., Ed. I. Karayiannis, *Acta Hort*. **488**.
7. FAO, (2005). FAO statistical database.
8. Guleryuz, M. (1991). The problem of rootstocks on fruit plant in Turkey and research on rootstock breeding in world. Turkey I. Nursery Symposium. Ministry Agriculture Pres, 273-285.
9. Murashige, T., Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant*. **15**: 473-497.
10. Rom, R.C. (1991). Apricot rootstocks. Prospective, Utilization and Outlook. IX. International Symposium on Apricot Culture. *Acta Hort*. **293** (2): 343-353.
11. Serebryakova, N.V. and Kalanova, A.I. (1978). The effect of water soluble vitamins on rose seed germination and rooting of cuttings. *Hort. Abst*. **44** (4): 5819.
12. Tuzcu, O., Kaplankıran, M., Yesiloglu, T. and Ozcan, M., (1991). The effects of germination and developing of different preservation methods on pecan (*Carya illinoensis*) seeds. Turkey First Nurseries Symposium. The Ministry of Agriculture Publication. 201-211.
13. Uslu, S., Guloglu, U., Mutlu, S., Pektekin, T., (1994). The Research Project of Rootstocks Characteristics for Some Apricot Varieties in Malatya. Malatya Fruit Research Institute, 46.