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Original Article

In vitro Propagation of Cataloglu Apricot (*Prunus armeniaca L.*) Cultivar Using Apical Node As Explant

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Summary. Apricot *(Prunus armeniaca L.)* is highly nutritious fruit and micro propagation of apricot plant has high economic value. The current study reports micro propagation of cultivar Cataloglu using apical node explants on Murashige and Skoog (MS) medium containing different combinations of BAP+NAA supplemented with 3% sucrose and solidified with 0.65% agar. The results showed a significant effect of concentrations of BAP+NAA on callus induction, shoot regeneration and their length. Rooting percentage was not affected on all shoots regenerated on any concentration of BAP+NAA. However, number of roots per explants and their length had clear implications of the concentrations of BAP+NAA used for regenerating shoots. Optimum shoot regeneration was noted on MS medium containing 1 mg/l BAP + 0.25 mg/l NAA; which were rooted on MS medium containing 1 mg/l IBA. The rooted plantlets were acclimatized in pots containing soil:sand (3:1) ratio under greenhouse conditions. It is concluded that the study will serve as reference for future apricot breeding studies and genetic transformation studies.

Keywords*:* Apricot *(Prunus armeniaca L.)*, Cataloglu, micro propagation

Introduction

The agriculture sector has strategic importance for all countries as it provides basic human needs. The apricot is an achene and an important fruit for agriculture. Apricot and its wild species are natural plants of a wide geographical region, from Central Asia to Northern China. Most scientists who study the origin of the apricot point to China and Central Asia as its homeland. Four subgenera are recognized in the *Prunus* genus (1). The apricot (*Prunus armeniaca L.*) has approximately 4 million tons annual production worldwide, and 800,000 tons in Turkey (2). Besides fresh and dry consumption of apricots, apricot kernels and sweet seeds are also used as snacks. Whereas apricot bitter seeds are used as raw material in cosmetics and pharmaceutical industries (3, 4), apricot kernel is mainly used for extraction of apricot oil, benzaldehyde, furfural, activated carbon, flavor extract, amygdalin and hydrocyanic acid (3). The stem of the apricot tree and its branches are used as fuel wood, and its leaves can be used for feeding animals. Apricot seeds can also be successfully used for biofuel and biogas production (5).

The apricot is also important for human health. It is rich in potassium, vitamin A and carotene, while it is deficient in sodium. That makes it an important nutrient for our diet. One of the most essential compounds of dried apricots for health is dietary fiber. The apricot has functional nutrients that strengthen defense mechanisms against free radical damage, delay aging, and prevent diseases, and can be recommended for a healthy and quality life (6).

In the current horticultural practice, the propagation of apricot is achieved only from seed, budding or grafting (7). Moreover, previous reports suggest difficulties in the rooting of the stem cuttings from mature

fruit bearing trees that prevent traditional propagation methods for propagation of these elite cultivars (8-12). To facilitate propagation of elite varieties and produce true to type plants micro propagation must be employed, that will facilitate regeneration of these cultivars in an accelarated way. There is little known on *in vitro* culture factors for regeneration from apical nodes of cv. Cataloglu. Therefore, the aim of the present study was to develop an *in vitro* micro propagation protocol and propose an alternative or complement to existing propagation techniques of the Cataloglu apricot cultivar of high economic value that may also be useful in future breeding studies with this cultivar.

Materials and Methods

Plant material

The newly growing shoot of the cultivar Cataloglu apricot (*Prunus armeniaca L.*) were used in this study, that were excised from newly growing shoots during spring season of 2015.

Sterilization

Glass materials (Petri dishes, Erlenmeyer flasks etc.), equipment (forceps, blades), and growth media were sterilized in an autoclave at 121 ℃ under 1.5 atm pressure for 20 minutes. After removing 3-4 cm twigs of cv. Cataloğlu growing at the Department of Horticulture, Inonu University, Faculty of Agriculture, Malatya, Turkey; they were surface sterilized using 30% hydrogen peroxide followed by 3×3 min rinsing with distilled water. Apical nodes were cultured in MS (13) culture media containing various concentrations of BAP and NAA. The apical node was excised from these twigs under aseptic conditions on and cultured on MS medium containing 5 explant per petri dish (100 ×10 mm).

Medium and culture conditions

After six days on MS medium, the apical nodes, the explants were cultured on the MS medium containing different combinations of 6-benzyl amino purine (BAP) and naphthalene acetic acid (NAA) for regeneration.

MS medium containing 1 mg/L indole-3-butyric acid (IBA) was used for rooting that was supplemented with 3% sucrose and the medium was solidified using 0.65% agar.

Double distilled sterilized water was used to prepare the growth medium and the pH was adjusted to 5.8 by using 1N NaOH and 1N HCl. BAP, NAA and IBA were dissolved in appropriate solvents, and stock solutions were prepared in the desired amounts and concentrations. Stock solutions were stored at +4 ℃.

Explant isolation from seedlings produced in vitro

Apical nodes explants were cut 1 cm in length, and were transferred to a regeneration medium (5 explants/petri dish). Trials were planned to have three repetitions. Hormones were added in concentration into MS media in 90×15 mm, sterile petri dishes, and petri dishes were incubated under a 16/8 light/dark period under 500 $\mu molm^{-2}s^{-1}$ fluorescence illumination at 25±2℃ in a plant growth chamber. All tissue culture studies were performed in a sterile, air-flow cabin.

Root formation in regenerated shoots

Once they reached a certain length, regenerated shoots were transferred to the rooting medium containing 1 mg/L IBA. Following root formation, the plants were transferred to MS medium to so as to provide opportunity to roots to reach a desirable size for transfer to pots containing 3 : 1 ratio of soil : sand for acclimatization.

Statistical analysis

The experiment was carried out using single factor, completely randomized design. Statistical analysis was performed using One way ANOVA with descriptive statistics for standard error with the help of IBM SPSS 21 computer software. Means were separated using Duncans multiple range test at $p<0.05$ level. All values shown in percentage were arcsine transformed before subjecting them to statistical analysis following Snedecor and Cochran (14).

Results

The results of the study showed significantly variable effects of variant BAP+NAA concentrations on apical nodes explant. Callus regeneration showed ir-

regular pattern of growth on MS medium containing 0.50 mg/l + variants of NAA and 1.00 mg/l + variants of NAA. Use of 0.5 or 1.00 mg/l BAP with or without 0.25-1 mg/l NAA promoted callus regeneration that never exceeded 33–34 % in each case. However, MS medium containing 0.5 or 1.00 mg/l BAP with 2.00 mg/l NAA promoted profuse callus regeneration. Maximum callus regeneration in each case was noted on 0.50 mg/l BAP + 2 mg/l NAA and 1 mg/l BAP + 2 mg/l NAA.

No shoot regeneration was noted on MS medium containing 1.00 mg/l BAP + 2.00 mg/l NAA. Percentage of shoot regeneration was not parallel to callus regeneration percentage. It ranged 10.00–51.00% and 14.00–70.00% on MS medium containing 0.50 mg/l BAP + 2 mg/l NAA and 1 mg/l BAP + 2 mg/l NAA respectively. Maximum shoot regeneration of 51.00% and 70.00% was on former and later was noted on MS medium containing 0.50 mg/l BAP + 1.00 mg/l NAA and 1 mg/l BAP + 0.25 mg/l NAA respectively.

Excluding no shoots on MS medium containing 1.00 mg/l BAP + 2.00 mg/l NAA, number of shoots per explant ranged 0.02±0.01-1.54±1.08 and 0.07±0.01-3.73±0.19 on MS medium containing 0.50 mg/l BAP + variants of NAA and 1 mg/l BAP + variants of NAA respectively. Maximum number of shoots per explant was noted on 1 mg/l BAP + 0.25 mg/l NAA. All other concentrations of BAP + NAA induced very low or marginal number of shoots per explant.

Shoot length showed two different behaviour on concentrations of 0.50 mg/l BAP + variants of NAA and 1.00 mg/l BAP + variants of NAA. Except MS medium containing 0.50 mg/l BAP + 1.00 mg/l NAA showing longest shoots of 2.38 ±1.16 cm long shoots, the shoots developing on all other explants never elongated beyond 1.36±1.02 cm in former case. On the later, MS medium containing 1.00 mg/l BAP showed longest shoots of 2.77 ±1.84 cm long shoots; the shoots developing on all other explants never elongated beyond 1.58±0.37 cm. Axillary shoot regeneration from the was only noted on calli exposed to air. No shoot regeneration was noted on portions of calli in direct contact with the culture medium.

Rooting was noted on all regenerants irrespective of the concentration of BAP +NAA used for regeneration (Table 2). However, the concentrations of BAP+NAA used in the experiment affected number of

Table 1: *Effect of different BAP and NAA concentrations on regeneration from apical nodes*

Hormone Treatments		Percentage (%) of callus formation	Percentage (%) of shoot regeneration	Number or shoots per explant	Shoots length (cm)
BAP (mg/L)	NAA (mg/L)				
0.50	0.00	21.00±1.08d	15.00±1.09d	0.21±0.09e	1.12±0.64d
0.50	0.25	40.00±2.02b	12.00±0.04d	0.72±0.03e	1.08±0.87d
0.50	0.50	14.00±1.04e	23.00±2.05c	0.47±0.07e	1.03±0.05d
0.50	1.00	33.00±3.06c	51.00±4.06a	1.54±1.08b	2.38±1.16b
0.50	2.00	87.00±5.01a	10.00±0.09d	0.02±0.01e	1.36±1.02d
1.00	0.00	26.00±2.01d	41.00±2.04b	0.97±0.26d	2.77±1.84a
1.00	0.25	10.00±1.01e	70.00±3.08a	3.73±0.19a	1.12±1.09d
1.00	0.50	19.00±2.03e	14.00±2.03d	0.07±0.01e	0.75±1.08d
1.00	1.00	34.00±2.05c	17.00±1.05d	1.23±0.92c	1.58±0.37c
1.00	2.00	50.00±4.07b	00.00±0.00e	0.00±0.00f	0.00±0.00e

All values shown in a single column with different letters are significantly different at 0.05 level of significance using Tukeys test
± Standard deviation of a minimum of three experimental repetitions.

Table 2. Effects of 1mg/l IBA on rooting of shoots regenerated on variants of BAP and NAA

Hormone Treatments		Rooting percentage (%)	Number of roots per explant	Root length (cm)
BAP (mg/l)	NAA (mg/l)			
0.50	0.00	100.00±00	0.71±1.19e	0.56±0.72f
0.50	0.25	100.00±00	1.86±0.93b	0.88±0.62d
0.50	0.50	100.00±00	0.65±1.11f	1.03±0.87e
0.50	1.00	100.00±00	2.43±1.34a	0.47±0.87g
0.50	2.00	100.00±00	1.52±1.32c	2.29±0.97a
1.00	0.00	100.00±00	0.73±0.49e	0.34±0.13h
1.00	0.25	100.00±00	0.43±0.34g	1.71±0.95b
1.00	0.50	100.00±00	1.13±0.62d	0.38±0.53h
1.00	1.00	100.00±00	0.96±1.23e	0.67±1.19 c

All values shown in a single column with different letters are significantly different at 0.05 level of significance using Tukeys test.

± *Standard deviation of a minimum of three experimental repetitions.*

roots per regenerated shoot and their length variably and showed significant differences ($p<0.05$) among them.

No shoots were noted on MS medium containing 1.00 mg/l BAP - 2.00 mg/l NAA; therefore these could not be treated with 1 mg/l IBA for rooting. The number of roots per shoots on rest of the regenerants on MS medium containing 0.50 mg/l BAP + variants of NAA and 1.00 mg/l BAP + variants of NAA ranged 0.65±1.11 - 2.43±1.34 and 0.43±0.34 - 1.13±0.62 respectively. Maximum number of 2.43 ± 1.34 roots per explant was noted on MS medium containing 0.50 mg/l BAP + 1.00 mg/l NAA.

Root length was also affected by the concentration of BAP + variants of NAA used for regeneration of shoots. Maximum root length was achieved on shoots regenerated on MS medium containing 0.50 mg/l BAP + 2.00 mg/l NAA. It was followed by significantly reduced ($P<0.05$) shoot length on MS medium containing 0.50 mg/l BAP + 0.50 mg/l NAA and 1.00 mg/l BAP + 0.25 mg/l NAA. Rest of the regenerants did not induce shoot length beyond 0.88±0.62 cm.

The best rooting shoots (regenerated on MS medium containing 0.50 mg/l BAP +2.00 mg/l NAA) were transfered to pots containing soil:sand (3 : 1) for acclimatization to external environmental conditions after two three weeks of culture. Out of 100 *in vitro*-regenerated plants transferred to pots only 83 plants survived and established under greenhouse conditions.

Discussion

The annual apricot production is approximately 4 million tons worldwide, and Turkey is a pioneer with 800,000 tons of annual production (2). Turkey is among the leading countries in dried apricot exports, and holds 80% of the market share. Apricots are mainly cultivated in Malatya (50%), Elazig, Erzincan, Sivas, Icel (Mut), Antalya, Hatay, Kars, and Igdir (15) in Turkey (16).

In the present study, apical nodes of the Cataloglu apricot cultivar, which has great economic value with respect to apricot production in Turkey, was used and the impacts of different BAP-NAA combinations were tested for its *in vitro* propagation for the first time. This protocol has the potential to serve as a good regeneration protocol for large-scale production of the Cataloglu apricot cultivar in breeding studies. Shoot regeneration using immature apricot cotyledons as explants are previously reported in a number of studies (17-20). In the present study, apical nodes were used as the explant source and MS culture media with 10 different combinations of BAP-NAA were used in the

trials. This result demonstrated that callus formation partially inhibits shoot regeneration and development.

The explant used in the study contained two injured portions on all sides that provided a way for uptake of nutrients and plant growth regulators for efficient absorption from the culture medium in agreement with Sarwar and Skirvin (21). Axillary shoot regeneration was noted on calli exposed to air (22). No shoot regeneration was noted on portions of calli in direct contact with the culture medium. Wang et al. (23) successfully obtained shoots by using hypocotyls developed from mature seeds. Wang et al. (24) conducted a study on mature apricot cotyledons and tested different combinations of TDZ (4 or 8 μM) and IBA (0.25 μM) and observed 67.2%, 56.8%, 66.7%, 46.3%, 66.7% shoot regeneration after a 2-week dark incubation. Compared to that study, shoot regeneration rates in the present study are relatively high. This difference may result from different concentrations of plant growth regulators, and differences in explants. Moreover, the choice of a different cultivar is an important cause in shoot regeneration, since the genotype of the cultivar significantly affects shoot regeneration (25). The current study represents the first study to use the apical node of the Cataloglu cultivar as the explant source.

The experimental results clearly showed positive role of BAP+NAA on shoot regeneration. However, optimum concentration of BAP+NAA was needed for regeneration of shoots. The results are not in agreement with Escalettes and Dosba (26). It is suggested that proposed concentrations of BAP+NAA increase metabolic activities in regenerating cells and the concentrations lower or higher than these concentrations are not suitable metabolically and hinder cellular growth and consequently shoot regeneration. The concentrations other than optimum concentrations hindered uptake of nutrients from the medium and the nutrient uptake was affected that also affected all parameters of growth in agreement with Pérez-Tornero and Burgos (25), who recommend use of BAP+NAA for shoot regeneration. The results further showed that an increase in NAA concentration was the best treatment to induce callus but it was not best treatment to induce shoot regeneration. Use of BAP singly was not suitable both for callus induction and shoot regeneration. However, optimum concentrations of BAP + NAA varied on 0.50 mg/l BAP+ variants of NAA and 1.00 mg/l BAP+ variants of NAA. This effect might be due to achieving a balance in endogenic and exogenic hormonal level of explant. This further suggests that both BAP and NAA concentrations in the regeneration medium compete for the active sites of the enzyme (27).

It is not always possible to promote root formation in media that favors shoot regeneration *in vitro*. Therefore, shoots should be transferred to a growth medium containing hormones that would stimulate root formation. In the present study, MS medium containing 1 mg/L IBA was used to stimulate root formation. The results of the study showed that rooting percentage was not affected by the concentrations of BAP+NAA used for regenerating shoots in the culture medium. However, concentration of BAP+NAA affected number of roots and their length. The results emphasize the role of shoot regenerating media on rooting. IBA has been recommended for rooting and calli regeneration of *in vitro* regenerated woody and herbaceous plant species (28) No problem was seen during acclimatization of regenerated and rooted shoots during acclimatization.

The present study may serve as a reference for more effective hormone concentrations and combinations for future studies on shoot regeneration in Cataloglu apricot. The study reports a procedure *in vitro* micro propagation of apricot cv. Cataloglu that include shoot regeneration, rooting and acclimatization. *In vitro* micro propagation of this cultivar using apical node has been described for the first time. The results of this study may serve as a base for developing clonal propagation technologies and genetic transformation for number of desirable traits. This procedure may be used in genetic transformation studies or breeding against a number of biotic and abiotic stresses or for any of the desirable apricot traits.

Bibliografia

1. Gülcan R, Mısırlı A, Eryüce N, Demir T, Sağlam H. Kayısı Yetiştiriciliği. 2001. İzmir. 212.
2. FAO. 2012. http://faostat.fao.org.
3. Asma BM, Mısırlı A. Kayısı Çekirdeği. Hasad, 2007; 55: 8-261.

4. Dwivedi DH, Ram RB. Chemical composition of bitter apricot kernels from ladakh. Acta Horticulturae, 2008; 8: 335-765.
5. Gezer I, Pektekin T, Aygül H, Polat H. Malatya Kayısı Raporu. Bilsam, 2009; Malatya.
6. Vardi N, Öztürk F, Gül M, Parlakpınar H, Çetin A, Otlu A. Metotreksat'ın neden olduğu intestinal hasar üzerine kayısının koruyucu etkilerinin araştırılması. Food and Chemical Toxicology, 2008; 46: 3015-3021.
7. Yıldırım H, Tılkat E, Onay A, Ozen HC. *In Vitro* Embryo culture of apricot, Prunus armeniaca L. cv. Hacıhaliloğlu. International Journal of Science & Technology, 2007; 2(2): 99-104.
8. Guleryuz M. The problem of rootstocks on fruit plant in Turkey and research on rootstock breeding in world. Turkey I. Nursery Symposium. Ministry Agriculture Pres, 1991; 273-285.
9. Uslu S, Guloglu U, Mutlu S, Pektekin T. The research project of rootstocks characteristics for some apricot varieties in Malatya. Malatya Fruit Research Institute, 1994; 46.
10. Eşitken A, Güleryüz M, Ercişli S. Embryo culture of hasanbey apricot cv. XIth International Symposium on Apricot Culture., Ed. I. Karayiannis Acta Horticulturae, 1999; 488.
11. Asma BM. Apricot growing. Evin Pres. Malatya. Turkey (In Turkish), 2000; 2: 243.
12. Asma BM, Ozturk K. Analysis of morphological, pomological and yield characteristics of some apricot germplasm in Turkey. Genetic Resources and Crop Evolution, 2005; 52: 305-313.
13. Murashige T, Skoog FA. Revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiologia Plantarum, 1962; 15: 473-497.
14. Snedecor GW, Cochran WG. Statistical methods. The Iowa State University Press, 1967; Ames Iowa.
15. Koçal H. Kayısı yetiştiriciliği. Meyvecilik Araştırma ºstasyonu Müdürlüğü Yayın, 2011; No:7.
16. Güner M, Vatandaş M, Dursun E. Bazı kayısı çeşitlerinde çekirdek kırılma karakteristiklerinin belirlenmesi. Tarım Bilimleri Dergisi, 1999; 5(1):95-103.
17. Goffreda JC, Scopel AL, Fºola JA. Indole butyric acid induces regeneration of phenotypically normal apricot (Prunus armeniaca L.) plants from immature embryos. Plant Growth Regulation, 1995; 17: 41-46.
18. Laimer Da Câmara Machado M, Da Câmara Machado A, Hanzer V, Weiss H, Regner F, Steinkeliner H, Mattanovich D, Plail R. Regeneration of transgenic plants of Prunus armeniaca containing the coat protein gene of Plum Pox Virus. Plant Cell Reports, 1992; 11: 25-29.
19. Lane WD, Cossio F. Adventitious shoots from cotyledons of immature cherry and apricot embryos. Canadian Journal of Plant Science, 1986; 66: 953-959.
20. Pieterse RE. Regeneration of plants from callus and embryos of 'Royal' apricot. Plant Cell Tissue Organ Culture, 1989; 19: 175-179.
21. Sarwar M, Skirvin RM. Effect of thidiazuron and 6-benzylaminopurine on adventitious shoot regeneration from leaves of three strains of `McIntosh' apple (Malus x domestica Borkh.) *in vitro*. Scientia Horticulturae, 1997; 68: 95-100.
22. Antonelli M, Druart P. The use of a brief 2,4-D treatment to induce leaf regeneration on Prunus canescens Bois. Acta Horticulturae,1990; 280: 45–50.
23. Wang H, Alburquerque N, Burgos L, Petri C. Adventitious shoot regeneration from hypocotyl slices of mature apricot (Prunus armeniaca L.) seeds: a feasible alternative for apricot genetic engineering. Scientia Horticulturae, 2011; 128: 457- 464.
24. Wang H, Petri C, Burgos L, Alburquerque N. Efficient *in vitro* shoot regeneration from mature apricot (Prunus armeniaca L.) cotyledons. Scientia Horticulturae, 2013; 160: 300-305.
25. Pérez-Tornero O, Burgos L. Different media requirements for micropropagation of apricot cultivars. Plant Cell Tissue Organ Culture, 2000; 63: 133-141.
26. Escalettes V, Dosba F. *In vitro* adventitious shoot regeneration from leaves of Prunus spp. Plant Science, 1993; 90(2): 201–209.
27. Pe´rez-Tornero O, Egea J, Vanoostende A, Burgos L. Assessment of factors affecting adventitious shoot regeneration from *in vitro* cultured leaves of apricot. Plant Science, 2000; 158: 61–70.
28. Ozdemir FA, Ortaeskinazi H. Antimicrobial activities of Acinos rotundifolius Pers. from extracts of hypocotyl and cotyledon node induced calli. Progress in Nutrition, 2016; 18(2): 184-189

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