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# Improved in vitro rooting of almond (*Amygdalus communis*) cultivar 'Nonpareil'

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**Abstract**

An efficient method was developed for rooting of almond (*Amygdalus communis* L.) cultivar, Nonpariel. Apical shoots of almond (*A. communis* L.) Nonpareil were cultured on Murashige and Skoog (MS) medium containing 1.0 $mgl^{-1}$ BA for micropropagation. After 3 weeks cultured elongated shoots were excised and their response to a range of rooting treatments investigated. Three experiments were conducted. (1) Elongated shoots were excised and their response to a range of rooting treatments investigated. Basal end of almond shoots were dipped into 1.0 g/l of IBA at different durations (10, 20, 30, 40, 50 seconds ) and (10,15, 20, 25, 30, 35 minutes) for rooting of almond shoots. Then, the dipped shoots were cultured on modified hormone free MS medium (1/2 and 1/4), respectively. (2) Shoots (2–3 cm in length) were excised and the basal end dipped in 2.5, 5.0, 7.5 and 10.0 mM IBA for 3 min, then placed in the modified half strength MS medium with 2% sucrose, 0,7 % w/v agar (Agar-Agar, Sigma) without plant growth regulators. Cultures were placed in the dark for 4 days prior to transfer to a 25 ± 2°C with 16 h photo period (40 µmol $m^{-2}$ $s^{-1}$) provided with mercury fluorescent lamps. (3) Shoots were cultured basic MS culture medium containing 2.5, 5.0, 7.5 and 10.0 µM IBA. The best root formation observed on the MS media (half strength) and dipped shoots 10, 15, 30 and 35 minutes at 1.0 g/l of IBA.

**Keywords:** *Amygdalus communis* L.cv. Nonpareil, Rooting, (PGRs) Plant Growth Regulators, *in vitro*
**Abbreviations:** BA-benzylaminopurine ($N^6$-benzyladenine), IBA (Indole butyric acid), MS - Murashige and Skoog medium.

## Introduction

The almond (*Prunus dulcis*, syn. *Prunus amygdalus*, or *Amygdalus communis*) is a small decidious tree belonging to the subfamily, Prunoideae of the family, Rosaceae. Almond (*Prunus dulcis* Mill.) is one of the major tree crops of the world (Kester et al., 1986). Conventional breeding of woody fruit species is slow and difficult due to the high levels of heterozygosity and long generation cycles (Ainsley et al., 2000). Almonds are one of the oldest commercial nut crops of the world; from the Middle and West Asia, it has diffused to other regions and continents which include the Middle East, China, the Mediterranean region and America (Ladizinsky, 1999). Besides its commercial use as a nut crop, the almond can be used for ornamental planting because it has beautiful flowers which are white or pale pink. Since it is a cross-pollinated species, a continuous genetic variation and heterozygous individuals have occured. Thus the homogenity decreased in the traditional orchards, which has led to very different fruit yield and quality (Gülcan, 1976; Küden, 1998). This, therefore, makes the plant tissue culture techniques more valuable for the clonal propagation of almond trees (Henry et al., 1992; Gomez and Segura, 1995). Many woody species are difficult to root through cuttings after the seedling-derived stage. Woody plants raise frequent propagation difficulties when using conventional techniques. Recently, different factors of *Prunus* micropropagation have been studied as the effect of subculture frequency (Grant and Hammat, 1999), the effect of different carbohydrates (Harada and Murai, 1996; Nowak et al., 2004), the comparison of different iron sources in the culture medium (Molassiotis et al., 2003), the effect of different combinations of growth regulators (Pruski et al., 2000), the application of mycorrhiza for pathogen protection, and the performance of micropropagated plants after their transfer to soil (Hammerschlag and Scorza, 1991; Hammat, 1999; Marín et al., 2003). Almond (*Amygdalus communis* Nonpareil.) is particularly difficult to root both *in vivo* and *in vitro*. *In vitro* rooting of this species is strongly genotype-dependent and successful only for some cultivars (Ainsley et al. 2001), while others only root after genetic transformation (Damiano et al. 1995). The rooting property of auxins and their importance in plant propagation have been widely recognized for long time (Thiman and Went, 1934). Hence, the necessity of exogenous auxin application to induce root formation in cuttings has been reported in many species e.g. sequoia (Berthon et. al., 1990), loblolly pine (Greenwood and Weir, 1994 ), juniper (Edson et. al., 1996), almond (Caboni et. al., 1997) and Eucalyptus (Fett-Neto et. al., 2001). The duration of auxin treatment can be a critical factor, since auxin is required for the induction of root primordia but it is often inhibitory for root growth at a later stage (De Klerk et. al., 1999). The choice of the auxin type and concentration can differ according to the specific culture conditions involved. The rooting of some woody species including *Prunus* can be also improved under darkness during the first week (Sriskandarajah *et. al.,* 1982; Rugini et. al., 1993; Caboni et. al., 1997). Currently, indole butyric acid (IBA) is the most widely used auxin to stimulate the rooting process in cuttings because of: 1) its high ability to promote root initiation (Weisman et. al., 1988) and 2) its weak toxicity and great stability in comparison to naphthalene acetic acid and indole-

**Table 1.** Effect of IBA (1 g/l) on the rooting percentage of almond shoots after 40 days culture on MS medium (values are the means of 3 replicates).

| Amount of IBA | Dip times of IBA | Rooting (%) | |
|---|---|---|---|
| | | ½ MS | ¼ MS |
| 1.0 g/L | 10 seconds | 0 | - |
| | 20 seconds | 0 | - |
| | 30 seconds | 0 | - |
| | 40 seconds | 0 | - |
| | 50 seconds | 0 | - |
| 1.0 g/L | 10 minutes | 10 | 0 |
| | 15 minutes | 10 | 0 |
| | 20 minutes | 0 | 0 |
| | 25 minutes | 0 | 10 |
| | 30 minutes | 30 | 0 |
| | 35 minutes | 20 | 0 |

**Table 2.** Effect of IBA (mM) on the rooting percentage of almond shoots after 40 days culture on MS medium (values are the means of 3 replicates).

| Culture medium | Amount of IBA | Dip times of IBA | Dark treatment | Rooting (%) |
|---|---|---|---|---|
| ½ MS | 2.5 mM | 3 minutes | 4 days | Fewer rooting |
| ½ MS | 5.0 mM | 3 minutes | 4 days | 0 |
| ½ MS | 7.5 mM | 3 minutes | 4 days | 0 |
| ½ MS | 10.0 mM | 3 minutes | 4 days | 0 |

3-acetic acid (Blazich, 1988; Hartmann et. al., 1990). Though auxins are widely used in the induction of rooting, little is known about their specific action or their interactions with other endogenous compounds (Gaspar et. al., 1997) .There are few reports of successful related to rooted and adventitious shoot regeneration from mature, elite hardwood trees (Liu and Pijut, 2008) ). There are few reports of successful related to adventitious shoot development from leaf and stem explants of *Amygdalus communis* L. cv. Yaltinski (Isıkalan et. al., 2010), effect of plant growth regulators on *in vitro* shoot multiplication of *Amygdalus communis* L.cv. Yaltsinki (Akbaş et. al., 2009) and *in vitro* Micropropagation of Almond *(Amygdalus Communıs* L.cv. Nonpareil) (Isıkalan et. al., 2008). The main objective of this paper was to find a reproducible method for the successful rooting of almond genotypes derived from a wide range of different *in vitro* culture treatments. We tested the effects of the induction pre-treatmentwith the auxin concentration, and mineral concentration of the culture medium rates on rooting of *in vitro* shoots of one almond cultivar. This *in vitro* study will help future workers on developing related manipulations.

**Materials and methods**

***Plant material***

Young offshoots were collected during the months of March–April from 7-years-old trees of *Amygdalus communis* L. cultivar, Nonpareil growing in the Botanical garden of the University of Harran, Şanlıurfa, Turkey. For establishment of *in vitro* shoot cultures, the leaves from young offshoots were eliminated and cutted 10-15 cm in length. These explants were washed with tap water for 5–10 minutes. Then, these were dipped in 70% ethanol for 30 second, surface-sterilized in 10% (w/v) commercial bleach solution (NaOCI) for 10 min, and rinsed five times with sterile distilled water (5 min per rinse). Steril apical shoots of almond (*Amygdalus communis* L.) Nonpareil (1.5–2.0 cm in length) were cultured on MS (Murashige and Skoog, 1962) medium containing 1.0 $mgl^{-1}$ BA for micropropagation (Işıkalan 2003). In conclusion, shoots were successfully propagated after two subcultures in the presence of 1.0 $mgl^{-1}$ BAP (Fig 1A,B,C). The medium was supplemented with 3% sucrose (w/v) and solidified with agar (0,7%, w/v, Agar- Agar, sigma). After 3 weeks cultured elongated shoots were excised and their response to a range of rooting treatments investigated. To root of *in vitro* shoots were conducted at three different applications. Each treatment contained at least 40 explants. In the first one, basal end of almond shoots were dipped 1.0 g/l of IBA at different times (10, 20, 30, 40, 50 seconds ) and (10,15, 20, 25, 30, 35 minutes) for rooting of almond shoots. Then, the dipped shoots were cultured on modified MS medium (1/2 and 1/4) free hormone respectively. The medium was supplemented with 2% sucrose (w/v) and solidified with agar (0,7%, w/v, Agar- Agar, sigma). In the second experiment, elongated shoots (2–3 cm in length) were excised and the basal end dipped in 2.5, 5.0, 7.5 and 10.0 mM IBA for 3 min, then placed on the modified half strength MS medium without plant growth regulators. The medium was supplemented with 2% sucrose (w/v) and solidified with agar (0,7%, w/v, Agar- Agar, sigma). Cultures were placed in the dark for 4 days prior to transfer to a 25 ± 2°C with 16 h photo period (40 µmol $m^{-2}$ $s^{-1}$) provided with mercury fluorescent lamps. In the third experiment, micropropagated shoots were cultured basic MS culture medium containing 2.5, 5.0, 7.5 and 10.0 µM IBA. The medium was supplemented with 3% sucrose (w/v) and solidified with agar (0,7%, w/v, Agar- Agar, sigma). In this study, All media were adjusted to pH 5.8 prior to autoclaving (121°C for 25 min), and *in vitro* cultures were maintained at 25 ± 2°C with 16 h photo period (40 µmol $m^{-2}$ $s^{-1}$) provided with mercury fluorescent lamps.

**Results**

***First experiment***

After 3 weeks in cultured elongated shoots were excised and their response to a range of rooting treatments investigated. The effects of two different MS concentrations ( 1/2 and 1/4) on the root formation of shoots were investigated. The first

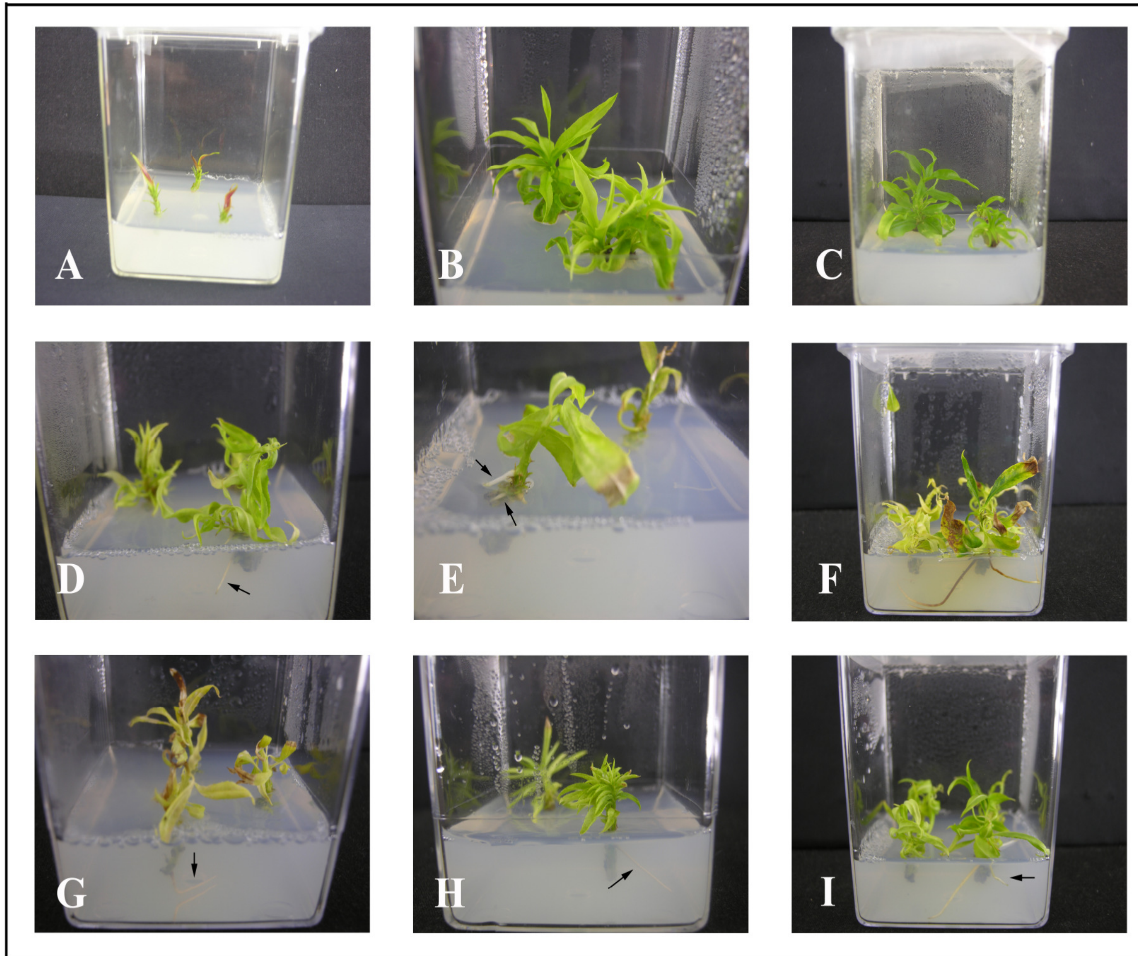


**Fig1.** *In vitro* rooting of *Amygdalus communis* L. cv. Nonpareil. (**A**) Aspect of shoot tips cultured on MS medium supplemented 1 mg/l BAP. (**B, C**) Multiple shoots grown on MS medium supplemented with 1.0 mg/$l^{-1}$ BAP. (**D**) Aspect root formation on a modified ½ MS medium from dipped shoots 10 minutes. (**E** )15 minutes (**F**) 30 minutes (**G**) 35 minutes at 1 g/l of IBA. (**H, I**) Aspect root formation on a modified ¼ MS medium from dipped shoots 30 minutes at 1 g/l of IBA.

root development was obtained at 10, 15, 30, 35 minutes dipped 1.0 g/l of IBA by using the ½ and ¼ MS media containing 20 g/l sucrose (Fig1D, E, F, G). After 40 days in culture, The best root formation occurred on the MS media (half strength) and dipped shoots 30 minutes at 1.0 g/l of IBA (Fig 1F). In the other parameters weren't observed root induction. By increasing of dipped times at 1.0 g/l of IBA, the fresh mass of leaves decreased and yellowish, too. Our results suggest that 1.0 g/l of IBA might be the 30 minute dipped effective for root induction and plant survival (Table 1). Between the use of ¼ and 1/2 modified MS significant differences weren't observed on root formation at the among shoot groups in view of mineral concentration of the culture medium ( Fig H,I). No root development was observed in any of the other treatments of IBA (10, 20, 30, 40, 50 seconds) for ½ MS and control groups.

***Second experiment***

Elongated shoots (3-4 cm in length) were excised and the basal end dipped in 2.5, 5.0, 7.5 and10.0 mM IBA for 3 min, then placed in the modified half strength MS medium without plant growth regulators. The rooting response was scored after 40 days of culture (Table 2). After 4 weeks in cultured all shoots be come dry and weren't observed root induction.

***Third experiment***

Without having each a pre-treatment, micropropagated shoots were cultured basic MS culture medium containing 2.5, 5.0, 7.5 and 10.0 µM IBA. The medium was supplemented with 3% sucrose (w/v) and solidified with agar (0,7%, w/v, Agar- Agar, sigma). The rooting response was scored after 40 days of culture (Table 3). After 4 weeks in culture, IBA did not stimulate adventitious rooting of the explants and rooting was very low in comparison to the control treatment. A high percentage of shoots on the rooting media containing the concentrations µM of IBA had shown symptoms of chlorosis and apex necrosis.

**Discussion**

The current study reports the development of a highfrequency rooting system from shoot explants of almond. Almond shoots cv Nonpareil obtained from *in vitro* conditions were used as material for rooting studies. No root development

**Table 3 .** Effect of IBA( µM) on the rooting percentage of almond shoots after 40 days culture on MS medium (values are the means of 3 replicates).

| Culture medium | Amount of IBA | Rooting (%) |
|---|---|---|
| 1/1 MS | 2.5 µM | 0 |
| 1/1 MS | 5.0 µM | 0 |
| 1/1 MS | 7.5 µM | 0 |
| 1/1 MS | 10.0 µM | 0 |

was observed in any of the other treatments of IBA (10, 20, 30, 40, 50 seconds) for ½ MS and control groups. Also, root development weren't observed in any of the other treatments of IBA (0.20.0,25. min anlamadım) for ½ and 1/4 MS. Liu and Pijut (2008) reported that the best results for rootion of the almond (*P. serotina*) was obtained from IBA dip. However, there also found that some shoot tips deteriorated after rooting. Our results are agrements with this study. In almond cv. Nonpareil, 1.0 mM IBA induced higher rooting frequencies than 0.5 mM (Ainsley *et. al*., 2001) but no other parameters were compared. In our study was compared with other parameters of this study. In our study determined that the root induction is considerably decreased when the IBA (2.5, 5.0, 7.5 and 10.0 mM) was dipped to basal end of shoots for 3 min. Our results are agrements with this study. Root elongation wasn't stimulated in any of the other treatments of IBA levels (2.5, 5.0, 7.5 and 10.0 mM ) for dipped 3 min on half strength media, however means were not significantly different. El Euch et. al., (1998) reported that in walnut, apical necrosis has been associated to a high sensitivity to IBA during root induction, possibly by the oxidation of phenolic compounds. Our results are agrements with this study. Similar observations were obtained for Nonpareil. In the present investigation. IBA played an important role as a plant growth regulator and had a significant effect on the average number of roots initiated apical shoots explant. Druart et. al., (1982) reported that darkness was more promotive to root formation of *in vitro* apple shoots at the period of root initiation. Beneficial effects of the dark treatment were found chiefly in difficult-to-root woody plants and have been demonstrated in many genera. In contrast, our results showed that cultures weren't observed root formation and cultures were placed in the dark for 4 days after dipped 2.5, 5.0, 7.5 and 10.0 mM of IBA. In *Prunus armeniaca*, apical necrosis on rooting has been prevented by using the auxin at low concentration (Murai et. al.,1997) or at high concentration for a short period of time (Pérez-Tornero and Burgos, 2000). Culture medium performance also depends on the genotype, thus, in almond AP medium performed better in the establishment of cultures of the cultivar Nonpareil, whereas MS medium was preferred for the cultivar Ne Plus Ultra (Channuntapipat, 2003). The objective of the present study was to determine the conditions required root formation and to facilitate regeneration of plantlets from shoots derived from *in vitro* cultures of the almond paper-shell varieties Nonpareil (Californian Papershell).

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