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# Effects of acetlysalicylic acid with indole-3-acetic acid on rooting and pigmentation in *Amygdalus* L.

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**Abstract.** Vegetative propagation is a key step, playing an important role in the succesful production of elite clones. The use of plant hormanes can increase the rroting capacity of cuttings. In this experiment, we investigated whether exogenously applied acetylsalicylic acid (ASA) with indole-3-acetic acit (IAA) (50, 100 mg/L) through the rooting medium could increase effects on Amygdalus spp or not. In the experiment, one year old semihardwood shoot cuttings were used. The highest callus formation was determined on the applied cuttings with 50 mg/L ASA with IAA in all groups. Rooting among the application groups in A. lycioides was observed around 10% in 50 mg/L ASA with IAA treatment group. Pigmentation contents in cuttings were found to be important in a time-dependent fashion.

**Keywords:** Rooting, acetylsalicylic acid, indole-3-acetic acid,callus

## 1. INTRODUCTION

Many species and cultivars of *Amygdalus* are produced for their edible fruit or as garden ornamentals, and a few species are also grown for their edible seeds [1]. *Prunus amygdalus* seedlings have been used for production since many centuries [2]. *P.amygdalus*is a nut tree, which has been grown in large areas in the world due to having climatic tolerance and having many microclimates suitable for almond growth [3]. In Turkey, the production budding and/or grafting are not common; and instead seed has been usually used for production [4]. The initiation and development of adventitious roots are vital processes for successful clonal propagation by cuttings.

Although rooting of several *Prunus* species has been reported, including apricot [5], cherry [6], peach [7], and plum [8], *in vitro* rooting of almond has proven difficult [9]. Reports describing rooting of adult almond explants are limited, and have primarily focused on hard shell cultivars grown in Europe [10, 11]. In Australia and the USA, paper shell types such as 'Nonpareil' are the main cultivars grown. Limited success has so far been achieved for the *in vitro* induction of adventitious roots with the premier paper shell cultivar 'Nonpareil' [12]. Nicotra and Pellegrini (1989) were reported that adventitious roots values given emitted higher than 30% only in 7 varieties (M 51, M 49, Ferragnes, M55, Genco, M50 and Texas) of almond's 26 varieties [13].

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Cuttings from some species, clones, or developmental phases within clones are competent for adventitious root initiation, while others are not [14]. Adventitious root formation is a requirement in any successful vegetative propagation program, many ecologically and economically important hardwood tree species have a low genetic or physiological capacity for adventititious root formation and are considered recalcitrant to routine commercial-scale vegetative propagation. However, successful propagation of difficult-to-root species can be achieved if the type of cutting (hardwood, softwood, or root) date of collection (seasonal growth development), stock plant or cutting manipulation (pruning, wounding etc.), rooting treatment (auxin type and concentration, rooting media) and greenhouse parameters (mist bed system, supplemental, lighting, temperature, etc.) are carefully considered [15].

Stimulation of root initiation, the second was the first practical use to be made of growth regulators [16]. Furthermore, the stimulation of adventitious roots by phenolic compounds has been reported for a number of plant species. Catechol, *p*-hydroxybenzoic acid, pyrogallol and salicylic acid stimulated root initiation in mung bean cuttings [17].

Salicylic acid (SA), a common plant phenolic compound, influences numerous physiological and biochemical process in plants. These include adventitious root initiation[18, 19],inhibition of ethylene biosynthesis [20], disease resistance [21], salt and osmotic stress [19, 22], ozone [23], growth and photosynthesis [22, 24], heat production [25], chilling tolerance [26].Thus more and more attention was paid to the action of SA, and as a result, SA has been proposed as a new plant hormone [27]. In tissue culture applications have emerged for SA or its artificial analogue acetylsalicylic acid (ASA) which has been found to be more effective in some systems [28]. Auxins have been shown to have the greatest effect on rooting [29-31]. Nevertheless, auxins have failed to promote root initiation or they had only a slight rooting effect in the case of hard-to-root olive cultivars [32, 33]. Treatment of cuttings with acetylsalicylic acid in combination with IAA can be of value in the propagation of woody plants [17].

In this study, we investigated the effects of ASA with IAA (50, 100 mg/L) on rooting and pigmentation in three species of *Amygdalus* (*A. trichamygdalus*, *A. orientalis* and *A. lycioides*).

## MATERIALS AND METHODS

### *Preparation of the plant samples*

One year old shoot cuttings of ten-year-old plants of *Amygdalus trichamygdalus*, *Amygdalus orientalis* and *Amygdalus lycioides* taken in November and December 2002 from Gündüzbey country of Malatya were used. The cuttings were prepared, 20-25 cm in length with 8-10 buds. The cuttings were washed for 24 hwith tap water. Basal ends of cuttings were soaked in 0, 50, 100, mg/L ASA+IAA dissolved in water for 24 hours. The treatments had three replicates which have ten cuttings each. The cuttings were grown in controlled climate chamber at 24±2ºC, 60±5% relative humidity, a photoperiod of 16 h, and a light intensity of 12.000 lux. Samples were grown perlite and with three-repetitions by using Hoagland solution [34]. The callus was recorded two months after planting.

### *Pigment extraction and determination*

In stem cuttings were analyzed chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll and carotenoid (Car) contents after ASA+IAA treatment. Duringthe extraction of the pigments,methods suggested byDe-Kok and Graham (1989) were used [35]. The absorbance values of the extraction process, were determinated (UV-1201V Shimadzu spectrophotometer) at 470, 645 and 662, nm according to Lichtenthaler and Welburn (1983) and total rates of Chl a, Chl b, total Chl and Car were calculated [36].

### *Statistical analysis*

Statististical analysis were performed using SPSS 10.0 for Windows. Duncan's Multiple Range Test was employed to determine the statistical significance of differences among the means [37].

## RESULTS

Application of ASA with IAA caused a significant increase in callus rate of *A. lycioides* in November and in December. At the same time a rooting of approximately 10% was occurred in the group applied 50 mg/L of ASA with IAA in November. As seen in Table 1, compared to control ASA with IAA treatment caused a marked decrease in callus rate of *A. orientalis* in November. On the other hand in *A. trichamygdalus* no such variations were recorded in November and in December (Table 1).

**Table 1** Calllus rate in the cuttings of *A. trichamygdalus*, *A. orientalis and A. lycioides* on a monthly basis.

| | | | Callus rate (%) | Rooting rate (%) |
|---|---|---|---|---|
| November 2002 | *A. trichamygdalus* | Control | 47.20±2.45$^{b}$ | ND |
| | | 50 mg/L ASA+IAA | 54.85±5.01$^{a}$ | ND |
| | | 100 mg/L ASA+IAA | 46.97±2.63$^{b}$ | ND |
| | *A. orientalis* | Control | 90.00±2.55$^{a}$ | ND |
| | | 50 mg/L ASA+IAA | 59.87±3.76$^{b}$ | ND |
| | | 100 mg/L ASA+IAA | 56.70±2.90$^{b}$ | ND |
| | *A. lycioides* | Control | 87.78±4.72$^{b}$ | ND |
| | | 50 mg/L ASA+IAA | 100.00±0.00$^{a}$ | 9.86±0.09 |
| | | 100 mg/L ASA+IAA | 93.63±5.53$^{b}$ | ND |
| December 2002 | *A. trichamygdalus* | Control | 40.00±0.05$^{b}$ | ND |
| | | 50 mg/L ASA+IAA | 50.00±0.05$^{a}$ | ND |
| | | 100 mg/L ASA+IAA | 40.00±0.05$^{b}$ | ND |
| | *A. orientalis* | Control | 40.00±0.05$^{b}$ | ND |
| | | 50 mg/L ASA+IAA | 50.00±0.05$^{a}$ | ND |
| | | 100 mg/L ASA+IAA | 40.00±0.05$^{b}$ | ND |
| | *A. lycioides* | Control | 83.33±5.77$^{b}$ | ND |
| | | 50 mg/L ASA+IAA | 93.33±5.77$^{a}$ | ND |
| | | 100 mg/L ASA+IAA | 80.00±3.64$^{b}$ | ND |

n=3
Within same column, means followed by letters are significantly different from each other ($P$<0.05) according to Duncan's test
ND=Not determined

Photosynthetic pigment content (Chl a, Chl b, total Chl and Car) were presented in Table 2. The highest Chl a amount was observedin *A. orientalis* groups which were treated with 50 mg/L ASA with IAA during the month of November as 5.08 µg/g fresh weight (FW) and December (6.20 µg/g FW). The highest Chl b amount, on the other hand, was determined in the same groups treated with 100 mg/L ASA with IAA during the month of December. In the species of *A. trichamygdalus* and *A. orientalis* applied 100 mg/L of ASA+IAA, the total chlorophyll amount increased noticeably.The highest carotenoid content was recorded in the groups applied 50 mg/L of ASA with IAA as 2.68 µg/g FW and 3.83 µg/g FW in November and December months respectively (Table 2).

**Table 2** Pigment content (µg.g$^{-1}$ fresh weight) in the cuttings of *A. trichamygdalus*, *A. orientalis and A. lycioides* on a monthly basis.

| | | | Chl a | Chl b | Total chlorophyll | Carotenoid |
|---|---|---|---|---|---|---|
| November 2002 | *A.trichamygdalus* | Control | 4.13±0.42$^{b}$ | 3.99±1.79$^{a}$ | 8.12±2.05$^{b}$ | 1.14±0.46$^{b}$ |
| | | 50mg/L ASA+IAA | 4.09±1.55$^{b}$ | 3.77±1,34$^{b}$ | 7.86±2.87$^{c}$ | 0.97±0.33$^{c}$ |
| | | 100 mg/L ASA+IAA | 4.78±0.94$^{a}$ | 4.01±1.42$^{a}$ | 8.79±2.33$^{a}$ | 1.46±0.60$^{a}$ |
| | *A. orientalis* | Control | 3.86±0.55$^{c}$ | 2.27±0.42$^{c}$ | 6.13±0.38$^{c}$ | 1.84±0.27$^{b}$ |
| | | 50 mg/L ASA+IAA | 5.08±1.23$^{a}$ | 3.04±0.41$^{b}$ | 8.12±1.34$^{b}$ | 2.68±1.28$^{a}$ |
| | | 100 mg/L ASA+IAA | 4.95±0.03$^{b}$ | 4.70±1.31$^{a}$ | 9.65±1.31$^{a}$ | 1.01±0.10$^{c}$ |
| | *A. lycioides* | Control | 2.97±0.15$^{b}$ | 2.87±0.24$^{a}$ | 5.84±0.34$^{a}$ | 1.95±0.06$^{c}$ |
| | | 50 mg/L ASA+IAA | 2.80±0.16$^{c}$ | 2.23±0.22$^{c}$ | 5.03±0.16$^{c}$ | 2.04±0.06$^{b}$ |
| | | 100 mg/L ASA+IAA | 3.21±0.48$^{a}$ | 2.56±1.10$^{b}$ | 5.77±0.68$^{b}$ | 2.22±0.08$^{a}$ |
| December 2002 | *A. trichamygdalus* | Control | 4.00±0.49$^{c}$ | 3.13±1.53$^{c}$ | 7.13±1.75$^{c}$ | 1.49±0.51$^{b}$ |
| | | 50 mg/L ASA+IAA | 4.59±1.43$^{b}$ | 3.38±1.01$^{b}$ | 7.97±2.15$^{b}$ | 1.83±1.26$^{a}$ |
| | | 100 mg/L ASA+IAA | 4.87±0.64$^{a}$ | 4.35±1.35$^{a}$ | 9.22±1.86$^{a}$ | 1.24±0.47$^{c}$ |
| | *A. orientalis* | Control | 3.54±0.54$^{c}$ | 2.46±0.55$^{c}$ | 6.00±0.44$^{c}$ | 2.05±0.19$^{b}$ |
| | | 50 mg/L ASA+IAA | 6.20±0.20$^{a}$ | 3.12±0.39$^{b}$ | 9.32±0.20$^{b}$ | 3.83±0.16$^{a}$ |
| | | 100 mg/L ASA+IAA | 4.95±0.04$^{b}$ | 4.89±0.09$^{a}$ | 9.84±0.13$^{a}$ | 0.93±0.04$^{c}$ |
| | *A. lycioides* | Control | 2.54±0.27$^{c}$ | 1.84±1.13$^{b}$ | 4.38±1.08$^{c}$ | 1.96±0.05$^{c}$ |
| | | 50 mg/L ASA+IAA | 3.03±0.32$^{a}$ | 2.07±0.29$^{a}$ | 5.10±0.21$^{a}$ | 2.04±0.11$^{b}$ |
| | | 100 mg/L ASA+IAA | 2.83±0.53$^{b}$ | 2.12±0.86$^{a}$ | 4.95±0.99$^{b}$ | 2.29±0.21$^{a}$ |

n=3
Within same column, means followed by letters are significantly different from each other ($P$<0.05) according to Duncan's test

## DISCUSSION

Many researches reported that rooting process in *Amygdalus* propagation was very difficult. Due to this reason alternative rooting forms was studied by the use of cutting exudation solution [38] or indicated plant growth regulators as indol-butyric acid (IBA) and naphthalene acetic acid (NAA), nevertheless it is necessary to determine the best concentration of these products specifically for *Amygdalus* L. [39, 40]. Antonopoulou et al. (2005) reported that the effect of vitamin riboflavin ($B_2$) on *in vitro* of the almond x peach hybrid clone GF 667 did not stimulate adventitious rooting of the explants and rooting was very low in comparision with the control treatment. Our results were determined difficult of rooting of *Amygdalus* spp. However, in this study, in ASA with IAA application groups were observed high callus formation and rarely root formation [41].

In addition to auxins, phenolic compounds either alone or in combination of auxins [17, 42] have also stimulated adventititous root formation in cuttings of several species. Singh (1993) [43]found that SA stimulated root formation in young shoots of ornamental plants and Li and Li (1995) reported the formation of adventitious roots on hypocotyl cuttings of mung beans [44]. SA had synergistically acted with IAA and promoted the root formation in mung bean cuttings, but had no effect on *Acer* cuttings [17]. SA combined with NAA synergistically promoted the root

number and root lengths of the cuttings of several *Populus* spp. Combined application of IBA with various concentration of SA significantly promoted the rooting of cuttings with respect to untreated ones. But the rooting was markedly low compared to effect of IBA (5 g $l^{-1}$) [45]. Although this effect was seemed to be in relation with the clonal differences and cutting time rather than concentration and treatment method [46]. In our study, reported that a rooting of approximately 10% was occurred in the group applied 50 mg/L of ASA+IAA in November in *A. lycioides*. The best callus formation was observed on the applied cuttings with 50 ppm ASA with IAA in all application groups (Table 1).

The effect of SA application was expected in view of earlier studies that showed increased or decreased photosynthetic pigments following SA application, depending on type of species or cultivar. For instance, Chandra and Bhatt (1998) observed that an increasing or decreasing effect of SA on chlorophyll content of cowpea (*Vigna unguiculata*) depends on the genotype [47]. In another study treatment with SA increased pigment contents in maize [22] and wheat [48] grown under normal or stress conditions [19]. Radwan et al. (2006) were reported that in all SA treatments, chlorophyll a/b ratio was higher than healty controls [49]. Considering phenols effect, the lower and the moderate concentrations of salicylic acid (50 and 100 mg/L) recorded the highest values of chlorophyll a, b and carotenoids [50].In the species of *A. trichamygdalus* and *A. orientalis* applied 100 mg/L of ASA with IAA, the total chlorophyll amount increased noticeably.The highest carotenoid amount was recorded in the groups applied 50 mg/L of ASA with IAA as 2.68 µg/g FW and 3.83 µg/g FW in November and December months respectively (Table 2). Our results obtained from this work agree with similar findings of other researchers [19, 46, 47].

In conclusion, exogenously applied ASA can influence a number of biological processes in plants [27]. The present research, in one year old shoot cuttings of *Amygdalus* spp. described effects on rooting and pigment content of ASA with IAA. In shoot cuttings of *A. lycioides* was seen to enhance adventitious rooting of ASA with IAA at 50 mg/L. The results confirmed that ASA with IAA promoted to callusing in application groups. Pigmentation changes occured in application groups, but there was no correlation between pigmentationrates and callusing. In the results of study callus were formed high proportion (%93-100) in treated groups, unfortunately no or very few new rooting (10%) was observed.

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