J. BIOL. ENVIRON. SCI.,
2010, 4(12), 143-148

Endogenous Promoters and Inhibitors in Olive Cuttings in Relation to Adventitious Root Formation

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ABSTRACT

Levels of endogenous rooting promoters and inhibitors were determined in olive cvs "Roghani"(high rooting ability), "Tokhmkabki" and "Konservalia"(low rooting abilities). Cuttings were prepared and rooted in greenhouse under intermittent mist after that, samples for each cultivar were taken 60 days after cuttings preparation. Thin layer chromatography was used for purification and identification of endogenous plant hormones (Indol-3-acetic acid (IAA), Abscisic acid (ABA), Gibberellins (GAs) and cytokinins). Results indicated that "Roghani" olive cuttings gave significantly higher rooting percentage than "Tokhmkabki" and "Konservalia". Results showed significant differences in IAA concentration in leaves of "Roghani", "Tokhmkabki" and "Konservalia" at sampling date. IAA concentration in "Roghani" leaves was significantly higher than "Tokhmkabki" and "Konservalia" cultivars. These results suggest that auxin was confirmed as limiting factor of root initiation. However, insignificant differences in ABA and cytokinins concentration were recorded between the 3 olive cultivars. But the high GAs concentration in "Tokhmkabki" and "Konservalia" leaves coincides with the low rooting percentages of the cuttings.

Key Words: Olive, Indole-3-acetic acid, Abscisic acid, Gibberellin, Cytokinin.

INTRODUCTION

Auxins have been used for many years to promote root initiation in cuttings. With many difficult-to-root plants, applied auxins fail to induce root initiation to a degree comparable with that induced on an easy-to-root variety of the same species, and in many instances difficult-to-root varieties which have been treated with auxins don't root as well as easy-to-root varieties (Avidan and Lavee 1978, Bartolini et al. 2008). Conflicting information is available on changes in naturally occurring indole-3-acetic acid (IAA) during the rooting process. In some cases, IAA levels in cuttings increase substantially either immediately after removal from the mother plants or after a short delay. In other cases, IAA decreases during the induction phase of adventitious rooting and then increases during the initiation phase (Noiton et al. 1992, Wiesman and Epstein 1987). Rooting was related to the presence or accumulation of free auxins in bases of cuttings. Free auxin increases in the rooting zone prior to rooting (Pio et al. 2005, Ayoub and Qrunfleh 2006). Other phytohormones such as abscisic acid (ABA) can enhance the effect of auxin on root formation. Some works on pea and tomato showed a decrease of ABA content when rooting occurred. This led to the hypothesis of an inhibitory control of ABA on root formation (Wood 1983). Of more than 120 different naturally occurring GAs, gibberellic acid (GA_3) is the only gibberellin that has been extensively studied in this respect. An inverse relationship between endogenous GA level and rooting ability was found in Jonathan apples (Takeno et al. 1983). With respect to root formation, cytokinins have typically been observed as inhibitors of this process (Ayoub and Qrunfleh 2006). IBA and BA acted synergistically or additively to improve rooting in cuttings of "Frantoi" and "Leccino" olive cultivars (Bartolini and Ministro 1981). However, kinetins, Zeatin were not active in inducing root formation (Nemeth 1979).

No rooting studies were conducted to correlate the rooting ability of the olive cuttings with the endogenous hormone in cuttings. Therefore, this research aims to study the level of the naturally occurring plant hormones in "Roghani", "Tokhmkabki" and "Konservalia" olive leaves in relation to rooting potential of the cuttings.

MATERIALS AND METHODS

Cuttings 10 cm long with 3-4 leaves were prepared from 3 olive cultivars, "Roghani"(easy-to-rooting), "Tokhmkabki" and "Konservalia"(difficult-to rooting). After that cuttings were treated for 5 seconds with 4000 mg L^{-1} indol-3-butyric acid (IBA) dissolved in a 20% ethanol aqueous solution for rooting; each treatment comprised 80 cuttings, divided in to 4 replicates of 20, randomly distributed on the rooting bed. Samples for each cultivar were taken 60 days after cuttings preparation (root primordium differentiation).

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The leaves were detached from the sub-terminal part of the shoots and were frozen immediately in liquid nitrogen, lyophilized, ground and stored in deep freezer (-18 ºC) until analysis. The procedures of Shindy and Smith (1975), Wood (1983) and Hofman (1990) with some modifications were used for extraction and purification of the naturally occurring indole-3-acetic acid, abscisic acid, gibberellins and cytokinins. TLC was used for further purification and preliminary identification of IAA, ABA, GA_3 and cytokinins (Takahashi 1986). Rooting percentage was calculated after 120 days cuttings preparation.

Data were statistically analyzed using MSTATC computer program. The analysis of variance was performed to determine significant differences. Mean separation was carried out by the Duncan's multiple-range test, with significance defined as $p \leq 0/05$.

RESULTS

The IBA treated "Roghani" cultivar presents a rooting of 66.25 %, while in the same treatment conditions the "Tokhmkabki" and "Konservalia" shows a 23.75, 1% value (Figure 1). Results showed significant differences in IAA concentration in leaves of "Roghani", "Tokhmkabki" and "Konservalia" at sampling date. IAA concentration in "Roghani" leaves was significantly higher than "Tokhmkabki" and "Konservalia" cultivars (18.89µg g^{-1}). There were no significant differences between "Tokhmkabki" and "Konservalia" for IAA concentration (Figure 2). These results suggest that IAA concentration in "Roghani" leaves play significant role in the rooting of the cuttings and may be a limiting factor in this respect. However, insignificant differences in ABA and cytokinins concentration were recorded between the 3 olive cultivars (Figure 3, 4). There were significant differences in GAs concentration between 3 olive cultivars. The highest (16.61µg g^{-1}) GAs concentration was in "Konservalia" olive leaves (Figure 5). Although, the correlation between GAs concentration in leaves in 3 olive cultivars and rooting percentage was negative, the general trend indicates that high GAs concentration coincides with the low rooting percentage in "Konservalia" and "Tokhmkabki" cuttings. These results indicate that GAs concentration in "Konservalia" and "Tokhmkabki" leaves probably had inhibitory effect on adventitious root formation.

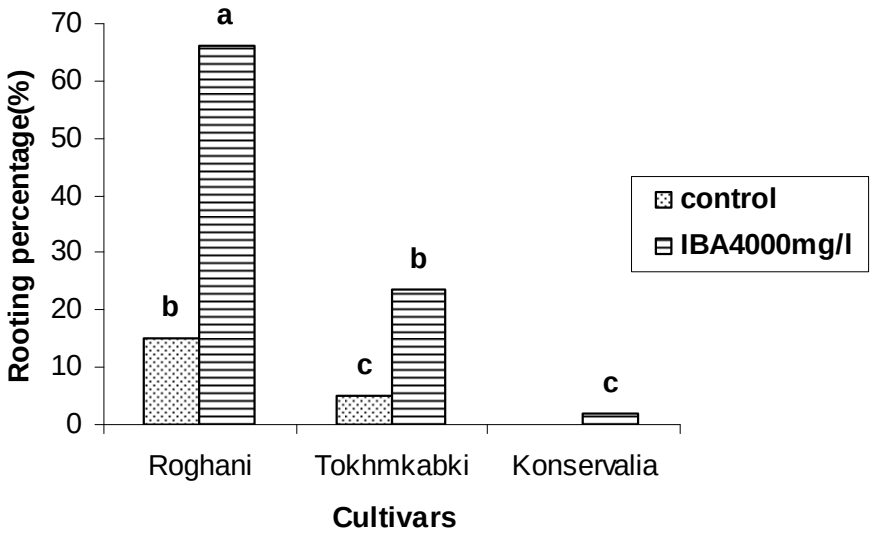

Figure 1. Rooting percentage in the cuttings after 120 days culture. Columns followed by the same letter do not differ significantly ($p \leq 0.05$).

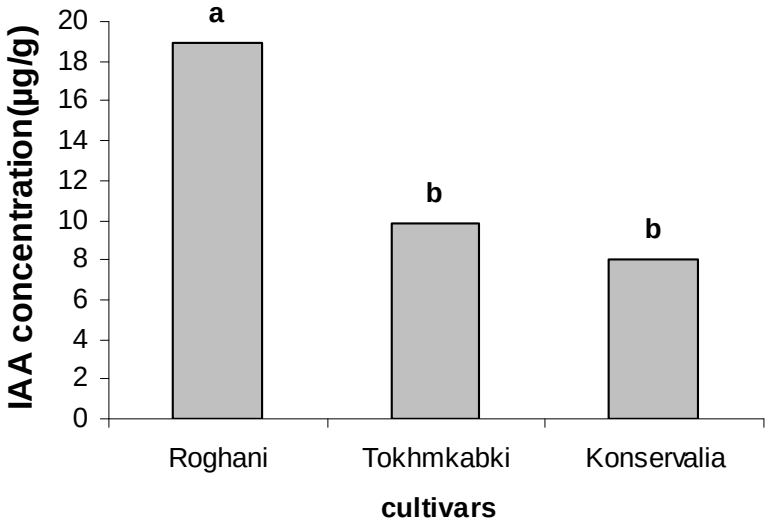

Figure 2. Endogenous IAA concentration in "Roghani", "Tokhmkabki" and "Konservalia" leaves in 60 days after cuttings preparation. Columns followed by the same letter do not differ significantly ($p \leq 0.05$).

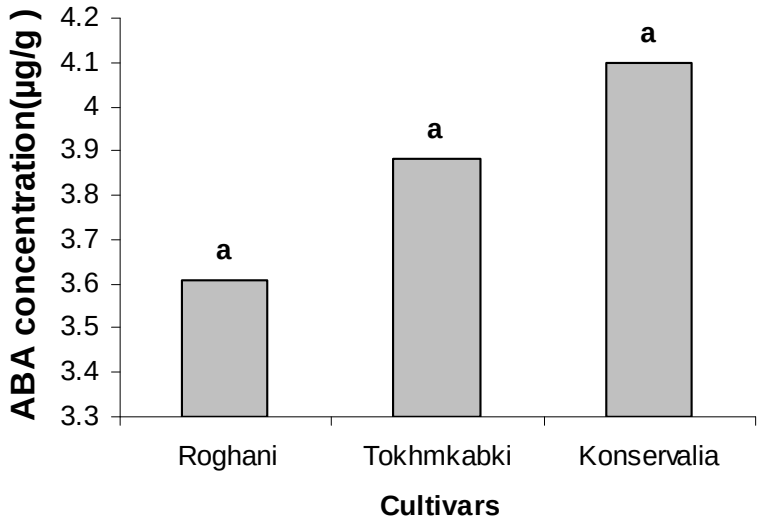

Figure 3. Endogenous ABA concentration in "Roghani", "Tokhmkabki" and "Konservalia" leaves in 60 days after cuttings preparation. Columns followed by the same letter do not differ significantly ($p \leq 0.05$).

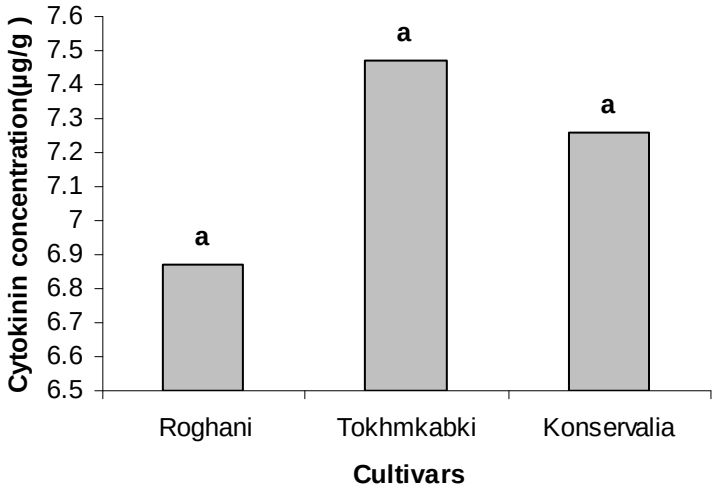

Figure 4. Endogenous Cytokinin concentration in "Roghani", "Tokhmkabki" and "Konservalia" leaves in 60 days after cuttings preparation. Columns followed by the same letter do not differ significantly ($p \leq 0.05$).

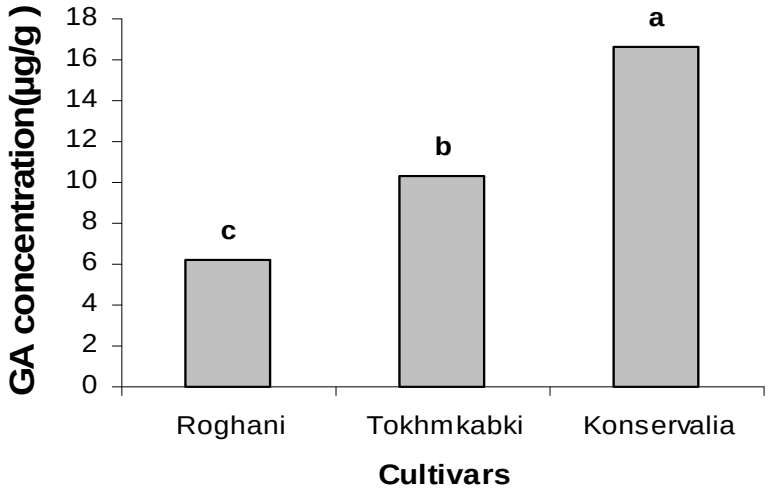

Figure 5. Endogenous GA concentration in "Roghani", "Tokhmkabki" and "Konservalia" leaves in 60 days after cuttings preparation. Columns followed by the same letter do not differ significantly ($p \leq 0.05$).

DISCUSSION

Olive propagation by cuttings was established 40 years ago. However, it has been found in various studies (Wiesman and Markus 2002, Serrano et al. 2002). That the rooting of many cultivars is very poor and there is wide variation in rooting ability of cuttings among the different cultivars (Avidan and Lavee 1978, Ullah and Awan 2004). As shown in (Figure 1) the majority of the cultivars showed a moderate or low rooting ability even in response to IBA treatment. Many researchers reported that was a positive correlation between IAA content and rooting ability of cuttings (Yong kweon and Ki sun 1996). Endogenous IAA promoted the induction of root primordia and increases the number of cells per root primodium by basipetal auxin transport (Yong kweon and Ki sun 1996). Auxin to be a limiting factor of root initiation in olive cultivars (Ayoub and Qrunfleh 2006). However, a positive correlation was noticed between the endogenous free auxin content and

the percent of rooting when auxin level was high at the time cuttings were made (Smalley et al. 1991). Also, rooting was related to the presence or accumulation of free auxins in the basis of herbaceous cuttings (Ayoub and Qrunfleh 2006). Promotive effect of auxin on rooting could be attributed to its role in stimulating cell division in the vascular cambium which leads to the formation of root primordial (Rahman et al. 2002). Therefore, it could be speculated that endogenous IAA play an important role in rooting of cuttings in olive. Accordingly Smalley (1991) ABA contents during rooting of stem cuttings of the *Acer rubrum* increased after the cuttings and decreased before rooting was observed. In this result no significant difference was recorded for ABA between the 3 olive cultivars during rooting. But also these results were disagreement with Ayoub and Qrunfleh (2006) who found that ABA concentration in "Nabali" olive buds and leaves may have a promotive effect on adventitious root formation. The probable inhibitory effect of GAs on adventitious root formation in "Tokhmkabki" and "Konservalia" is in agreement with Bartolini and Ministro (1981) who reported an inhibitory effect of GAs on rooting of "Frantoio" and "Leccino" olive cultivars, and with Takeno et al. (1983) who found an inverse relationship between GAs and rooting ability of apple cuttings. It was suggested that GAs acts by blocking the organized cell division that initiate the formation of root primordial. The inhibitory effect of GAs on rooting percentage of the cuttings was more pronounced in "Konservalia" than in "Tokhmkabki" olive.

In this result no significant difference was recorded for Cytokinins between the 3 olive cultivars during rooting and this result is in agreement with Ayoub and Qrunfleh (2006) who reported that no consistent relationship can be established between cytokinins concentration in buds and leaves of "Nabali" and "Raseei" cultivars and the rooting percentage of the cuttings and Nemeth (1981) who found that cytokinins were not active in inducing root formation in fruit tree rootstocks.

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