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Research article

# Improving the adventitious rooting ability of hard-to-root olive (*Olea europaea* L.) cultivar cuttings through inhibiting strigolactone biosynthesis

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**Abstract**

Strigolactones (SLs) are synthesized in roots and control plant development. As phytohormones, SLs regulate plant architecture, including roots. Recently, the inhibiting effects of SLs on adventitious rooting have been identified. Olive (*Olea europaea* L.) is consumed for oil and table in Mediterranean countries and is an economically important crop. Turkey is one of the countries with the highest olive production. Olive has mostly propagated asexually via cuttings, however, the rooting capacities of some agriculturally important olive cultivars are very low. Indole Butyric Acid (IBA) is commonly used to promote the rooting of olive cuttings, however, it can be inadequate. Ayvalık is an easy-to-root cultivar and one of the most common cultivars grown for oil production and Domat is a hard-to-root cultivar in which IBA is insufficient for inducing rooting. In our study, the effects of synthetic SLs *rac*-GR24 and SLs biosynthesis inhibitor TIS108 on the rooting ability of olive cuttings were investigated. As a result, the adventitious rooting ability was increased when a hard-to-root cultivar was treated with TIS108, indicating a promising future for olive-cutting rooting. Therefore, our study will provide potentially new tools for propagation strategies using SLs in fruit trees.

***Keywords:*** *Cutting rooting; GR24; olive; strigolactones; TIS108*

## 1. Introduction

Strigolactones (SLs) are carotenoid-derived plant metabolites and known to be germination stimulants for parasitic plant seeds for a long time (Cook et al., 1966). Then, they are found to be involved in the mutual interaction with arbuscular mycorrhizal fungi (Akiyama et al., 2005) and involved in the inhibition of lateral bud growth (Gomez-Roldan et al., 2008; Umehara et al., 2008). As endogenous phytohormones, SLs control plant architecture. Up to date, lots of studies have been carried out about SLs also affect root growth, primary root length, lateral root formation, root-hair elongation, adventitious rooting, root tip anatomy, stem elongation, leaf expansion in *Arabidopsis thaliana*, maize, petunia, rice, *Medicago truncatula*, *Vitis vinifera*, and *Solanum lycopersicum*. (Stirnberg et al., 2002; Snowden et al., 2005; Kapulnik et al., 2011; Ruyter-Spira et al., 2011; Arite et al., 2012; Guan et al., 2012; Kohlen et al., 2012; Rasmussen et al., 2013; de Saint Germain et al., 2013; De Cuyper et al., 2015; Santoro et al., 2020; Xu et al., 2021).

Olive (*Olea europaea* L.), one of the oldest cultivated trees in the Mediterranean Region (Rugini, 1986; Zohary et al., 2012) is commercially important since olive oil and table olive are consumed quite a lot in the Mediterranean diet. Therefore, olive production is one of the important agricultural sectors that have the potential for high income. Olive can be propagated by cuttings, grafting, or in vitro techniques, however, propagation via cuttings is the most widely used method in many countries (Fabbri et al., 2004).

Olive has generally propagated asexually via cuttings in a

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humid environment since the production of seeds is slow. However, the rooting capacities of some agriculturally important olive cultivars are very limited and can only be propagated by inoculation (Fabbri et al., 2004; Cetintas Gerakakis and Ozkaya, 2005). The exterior and interior factors that affect rooting capacities such as time of planting, size of cuttings, and properties of rooting media were reported (Cetintas Gerakakis and Ozkaya, 2005). Ayvalık is one of the most common cultivars grown in Turkey for oil production and is an easy-to-root cultivar (ER). The Olive Ayvalık cultivar is cultivated for its high oil quality (Isfendiyaroglu et al., 2009) and comprises 19% of the olive trees in Turkey (Kiralan and Bayrak, 2013).

Many studies revealed that auxins have the best effect on adventitious rooting (Fabbri et al., 2004). The olive cuttings are usually treated with Indole Butyric Acid (IBA) to promote rooting and the concentrations of plant growth regulator treated also affect the rooting abilities (Serrano et al., 2002). However, for a hard-to-root cultivar (HR) Domat, which bears large fruits, IBA is insufficient for inducing rooting (Cetintas Gerakakis and Ozkaya, 2005; Turkoglu and Durmus, 2005).

Recent studies on SLs are mostly carried out on model organisms, such as Arabidopsis or rice. However, little is known about the effects of SLs on fruit trees. Olive is an economically important crop in Mediterranean countries, and therefore, we investigated the effects of *rac*-GR24 as an SL analog and TIS108 as an SL inhibitor (Ito et al., 2013) on olive ER and HR cuttings. Finding a solution for rooting problems of HR cultivars is so important since grafting techniques are quite expensive. As a result, this study shows that TIS108 usage for HR olive cultivars provides new tools for cutting propagation strategies of fruit trees.

## 2. Materials and methods

Olive is mostly propagated via cuttings (Fabbri et al., 2004). For this reason, we aimed to determine the effects of *rac*-GR24 and TIS108 applications on the rooting ability of cuttings. We used the ER and the HR cuttings (Cetintas Gerakakis and Ozkaya, 2005).

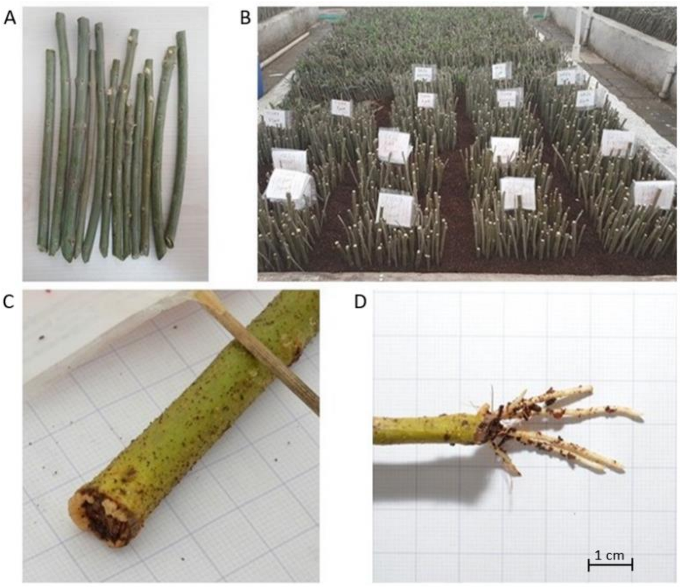


**Fig. 1.** A) Olive Cuttings prepared from olive trees, 25-30 cm each. B) Cuttings treated with *rac*-GR24 and TIS108 and planted in growing blocks. C) Callus formation on stem cuttings. D) Root formation on stem cuttings.

For each cultivar, 700 cuttings were tested (Fig. 1A). We treated cuttings with 1, 5, and 10µM *rac*-GR24 or TIS108 (Strigolab, Turin, Italy), and non-treated control groups were also included. Thus, for each treatment group, we had 100 cuttings for each cultivar.

For the applications, the basal pieces of both two olive cultivar cuttings were dipped into the plant growth regulator solutions (1, 5, and 10µM) one by one for 5 seconds and then planted into growing blocks that have a high water-holding capability (Fig. 1B). The cuttings were held in greenhouse conditions (25°C, 90-95% humidity) in the Edremit Directorate of Olive Production Station (Edremit, Balıkesir). Ten weeks later of the treatment, the cuttings were evaluated in terms of the formation of both callus and roots since the callus formation from the wounded parts is the first stage of adventitious rooting of cuttings (Fig. 1C-D).

## 3. Results

We investigated the callus formation and rooting ability of ER Ayvalık and HR Domat cuttings in the presence of either *rac*-GR24 or TIS108.

At the end of the 10 weeks, 46% of explants belonging to the ER control group produced callus (Fig. 2). In the *rac*-GR24 treatment groups, callus production varied from 30 to 36%, while TIS108 induced callus in 36 to 57% of the explants for the ER. In all application groups for *rac*-GR24 in the ER, the callus formation rates are lower than the control group while the 5 µM TIS108 group in which the callus formation was increased. However, only 9% of the explants belonging to the control group of HR-produced callus (Fig. 2). In the *rac*-GR24 treatment groups, callus production varied from 4 to 12%, while TIS108 induced callus 2 to 16% of the explants for the HR. For *rac*-GR24 applications, only the 10 µM applied group showed a slight increase in callus formation compared to the control and for TIS108 applications both 5 and 10 µM applied groups showed an increase in callus formation and this increase is compatible with the dose increase. According to these data, the best results for ER group were achieved with 5 µM TIS108 application (57%) and for HR cultivar with 10 µM TIS108 application (16%).

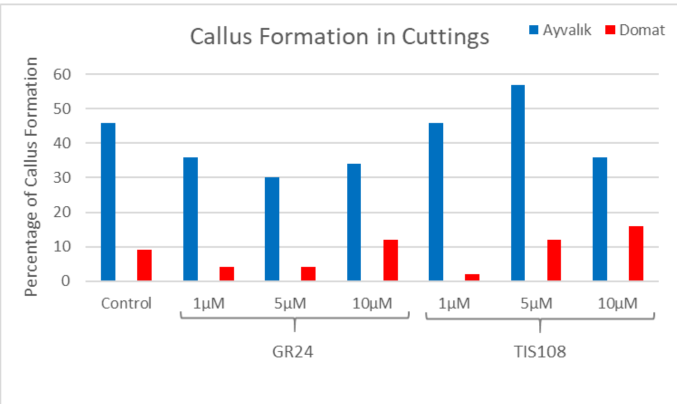


**Fig. 2.** Callus formation percentages of ER and HR olive cultivars

Afterwards, the cuttings which have roots were counted. At the end of the 10 weeks, the rooting rate was 1% in the control group of the ER (Fig 3). In the *rac*-GR24 treatment groups, rooting rates varied from 0 to 2%, while TIS108 induced rooting of 4 to 8% of the explants for the ER. For *rac*-GR24 applications, only the 10 µM *rac*-GR24 applied group did not show any roots. For HR, the rooting rate was 1% in the control group (Fig. 3). In the *rac*-GR24 treatment groups, rooting rates varied from 1 to 4%, while TIS108 induced rooting of 0 to 3% of the explants for the HR. For TIS108 applications, while 1 µM application did not show any roots, 5 and 10 µM applied groups showed increased rooting compared to the control group. According to these results, TIS108 is promising for inducing callus production and rooting of cuttings for both ER and HR olive cultivars.

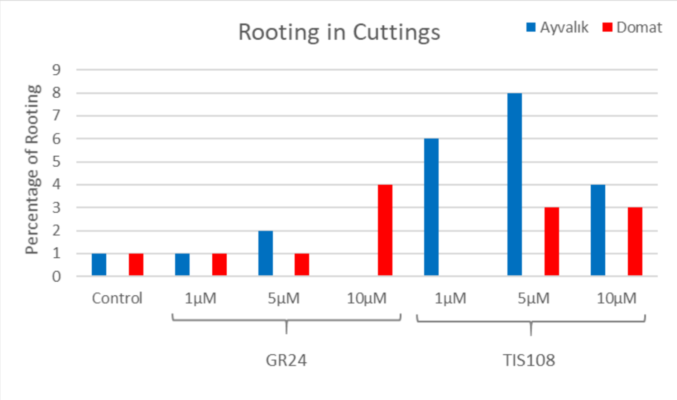


**Fig. 3.** Rooting percentages of ER and HR olive cultivars.

## 4. Discussion

The rooting abilities of some economically important olive cultivars are very limited. Therefore, we determined the effects of *rac*-GR24 and TIS108 on cuttings of HR and ER olive cultivars. SLs have been shown to suppress adventitious rooting in plants such as tomato, *A. thaliana*, and pea (Kohlen et al., 2012; Rasmussen et al., 2013). Similarly, in this study *rac*-GR24 suppressed both callus formation and adventitious rooting in cuttings of ER. For HR, 1 and 5 µM *rac*-GR24 applications could suppress callus formation and root formation. It is unclear whether this suppressive property of SL is direct or indirect (Kohlen et al., 2012). This feature may arise because of the shortage of the transport of auxin hormone, which promotes adventitious rooting, to the lower parts of the plant. (Crawford et al., 2010; Shinohara et al., 2013). Besides, in *A. thaliana* and pea, it was stated that the decrease in cell division or differentiation in the tissue expected to form adventitious roots, indicates that SL restricts organogenesis (Rasmussen et al., 2013).

For ER, 10 µM *rac*-GR24 application suppressed rooting completely while the same dose increased rooting for HR. For TIS108 applications all groups showed an increase in adventitious rooting for ER and HR except the 1 µM TIS108 application group for HR. Increased adventitious rooting was also observed in petunia, tomato, *A. thaliana*, and pea SL mutants (Kohlen et al., 2012; Rasmussen et al., 2013). According to these results, a more detailed analysis might be required to explain the effects of TIS108 for adventitious rooting in HR.

In conclusion, finding a solution to the rooting problem in hard-to-root olive cultivars will replace the very expensive grafting technique and pave the way for the development of less costly and easier methods. Enlightening the usage areas of SLs in woody plants will provide new opportunities in breeding, propagation, or productivity programs. Besides, in this study, the SLs inhibitor TIS108 increased the callus production and rooting ability of cuttings for HR cultivar, which is promising for quitting the expensive inoculation technique.

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**Conflict of interest:** The authors declare that they have no conflict of interests.

**Informed consent:** The authors declare that this manuscript did not involve human or animal participants and informed consent was not collected.

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