

## Endogenous Soluble Sugars, Starch Contents and Phenolic Compounds in Easy - and Difficult – to – Root Olive Cuttings

Elham-Asmoshtaghi\*, Ali Reza-Shahsavari

Department of Horticultural Science, College of Agriculture, Shiraz University, Shiraz, IRAN

### ABSTRACT

This study was carried out to elucidate the relationship between the monthly changes of rooting ability and inner condition of cuttings, such as soluble sugars, starch contents and the total phenolic content in cuttings of olive, and to determine the great differences in the rooting potential of cultivars and clones within a species were shown in many cultivars of olives. Several factors, at biochemical and molecular levels, interact in the formation and development of adventitious roots. The formation of adventitious root is an essential process in propagation of olive tree. The ability of rooting is critical in several strongly recalcitrant olive cultivars. The availability and mobilization of carbohydrates towards the base of cuttings appear can be major factors related to rooting of olive cuttings. Although "Roghani" cuttings shows a maximum soluble sugars and starch contents at the beginning of the experiment. But a completely different situation was found 60 days later. Soluble sugars were higher in all of the cultivars in comparison with at the beginning of the experiment. There were significant differences between 3 cultivars in the total phenolic content and the highest phenolic compounds were found in "Konsrvalia" after 120 days and the lowest phenolic compounds were found in "Roghani" cultivar.

**Key Words:** Olive, Rooting ability, Semi-hardwood cuttings, Sucrose, The total phenolic content

### INTRODUCTION

Olive (*Olea europaea* L.) has been propagated mainly by cutting. Stem cuttings are the important means of vegetative propagation in horticultural industry for mass production within a short time, but great differences in the rooting potential of cultivars and clones within a species were shown in many cultivars of olive (Dauod et al. 1989, Fernandes Serrano et al. 2002, Pio et al. 2005).

Some one showed that in many species, endogenous specific rooting factors determine the rooting potential of the cultivars. These factors were found to be synthesized in the leaves and buds and translocated to the base of the cutting. In many woody and herbaceous species, a negative correlation was found between the level of growth inhibitors and rooting potential (Caballero1979). Carbohydrates have been considering optimal markers since they are the main energetic resource during the rooting. The role carried out from carbohydrates during the rooting is controversial, but several reasons can explain their behavior. The levels of total carbohydrates and starch in the cuttings are positively related with the rooting but not through one cause-effect relationship (Delrio et al. 1991, Rahman et al. 2002).

Many experiments were carried out to clarify the seasonal changes of rooting ability and to manifest relationship between rooting ability and endogenous growth regulators extracts from cuttings. They indicated that cutting extracts of time in the year when cuttings showed high rooting percentages showed the root promoting effect and might contain auxin-like substances and rooting co factors (Fadl and Hartman1976). Also, the rooting capacity of many cuttings has been correlated with their carbohydrates content. The endogenous phenolic compounds have different effects especially on rooting of cuttings. In addition to plant hormones, enzymes and the total phenolic content play a significant role in internal control mechanism of rooting in olives. Both flavonoid and phenolic compounds from olive leaf are known to have diverse biological activities and may also be responsible for the pharmacological actions of olive leaf or, at least, for synergistically reinforcing those actions (Artajo et al. 2006, Bartolin et al. 2008). This study was carried out to elucidate the relationship between the monthly changes of rooting ability and inner condition of cuttings, such as soluble sugars, starch contents and the total phenolic content in cuttings of olive, and to determine the great differences in the rooting potential of cultivars and clones within a species were shown in many cultivars of olives.

### 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). Cuttings were treated for 5 seconds with 4000 mg L<sup>-1</sup> 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. For analyses,

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\* Corresponding author: emoshtaghi11@gmail.com

each treatment comprised 80 cuttings, divided in to 15 cuttings for 3 times of culture (0, 60, and 120 days after cuttings preparation). After 120 days rooting and shooting were evaluated. Leaves were sampled at the beginning of the experiment and 60 and 120 days after that, for soluble sugars, starch contents and the total phenolic content. Sampled were frozen immediately in liquid nitrogen, lyophilized, ground and stored in deep freezer (-18 °C) until analysis. Soluble sugar and starch were determined by the Duboifh et al. 1956. The total phenolic content was determined by the Folin-Ciocalteu. Folin-Ciocalteu reagent is the classic reagent recommended for the total phenols (Tsimidou et al. 1992). Data were submitted to statistical analysis using Duncan's multiple-range test, with significance defined as  $p \leq 0/05$ .

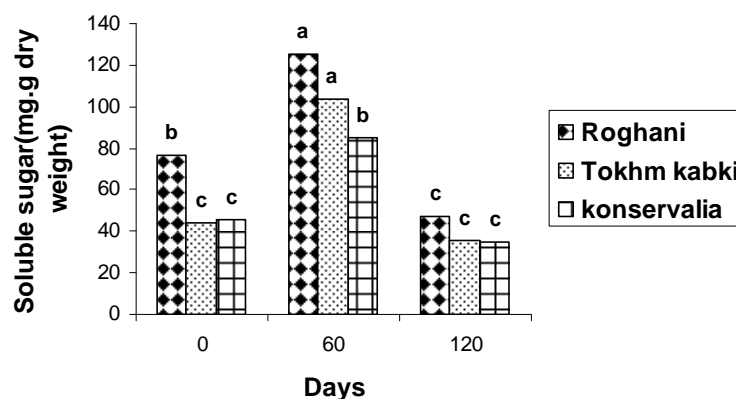
## RESULTS

Olive rooting is apparently related to the genotype: in fact, the three genotypes used in this study have shown different rooting abilities. 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 (Table.1).

**Table 1.** Rooting percentage in the cuttings after 120 days culture. Mean separation within columns by Duncan 's test

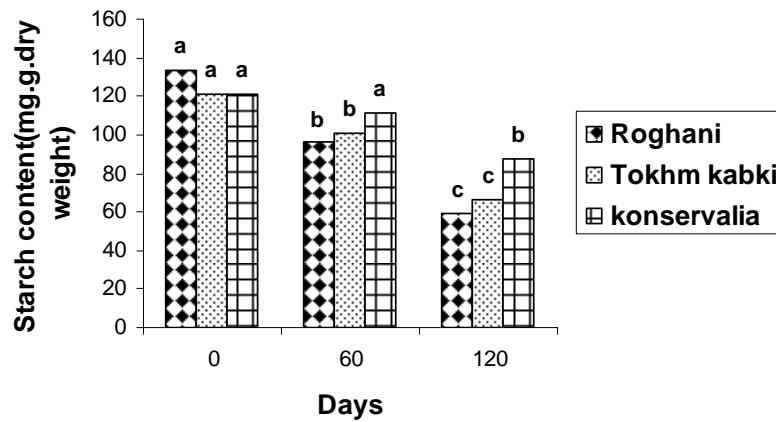
Treatments	Cultivars		
	Tokhmkabki	Roghani	Konservalia
0	5% c	15% b	0% c
IBA 4000 mg L <sup>-1</sup>	23.75% b	66.25% a	1% c

The results of soluble sugars expresses in mg g<sup>-1</sup> of dry weight in the 3 cultivars show different initial availabilities. Although "Roghani" cuttings shows a maximum soluble sugars and starch contents at the beginning of the experiment but also there were no significant differences between 3 cultivars for starch contents. Completely different situation was found 60 days later. Soluble sugars were higher in all of the cultivars in comparison with at the beginning of the experiment. Therefore starch contents were decreased after 60 days. The greatest soluble sugars (125.6 mg g<sup>-1</sup>DW) occurring in "Roghani" cultivar (Figure.1).



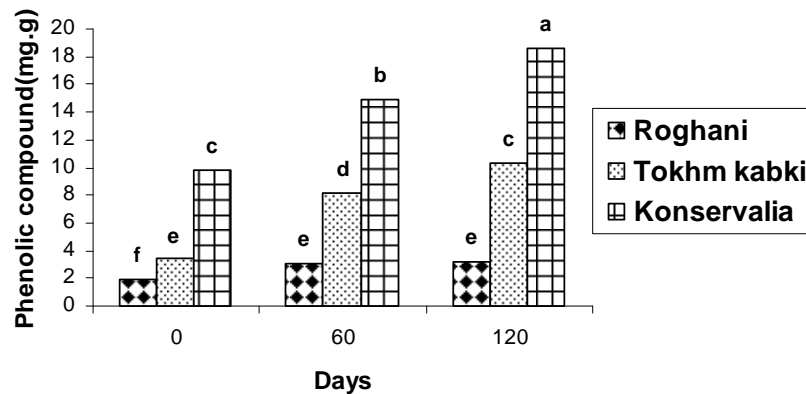
**Figure 1.** Soluble sugar (mg g<sup>-1</sup>DW) during the rooting culture (0-120 days) of 3 olive cultivars. Columns followed by the same letter do not differ significantly ( $p \leq 0.05$ ).

Soluble sugars and starch contents a subsequent diminution at the end of the experiment and there were no significant difference between 3 cultivars for soluble sugars at the end of the experiment (Figure.1, 2).



**Figure 2.** Starch content (mg g<sup>-1</sup>DW) during the rooting culture (0-120 days) of 3 olive cultivars. Columns followed by the same letter do not differ significantly (p≤0.05).

The amount of phenolic compounds showed variation among months and was higher after 120 days. There was significant differences between 3 cultivars in monthly changes of phenolic compounds and the highest phenolic compounds were found in "Konservalia" (18.56) after 120 days and the lowest phenolic compounds were found in "Roghani" cuttings (Figure.3). Our study showed that there was clear relationship between rooting and the amounts of the phenolic compounds, soluble sugars and starch content in the leaves of the olive cuttings.



**Figure 3.** Phenolic compounds (mg g) during the rooting culture (0-120 days) of 3 olive cultivars. Columns followed by the same letter do not differ significantly (p≤0.05).

## DISCUSSION

The availability and mobilization of carbohydrates towards the base of cuttings appear can be major factors related to rooting of olive cuttings. The soluble sugars increased in all the cuttings until 60 days, and afterwards decreased. The high carbohydrate, fructose and glucose contents, and C/N ratio during the growing season coincided with the high rooting in olive cuttings. Therefore, it was considered that fructose and glucose are used as energy source for cell division in rooting (Reuveni and Raviv 1981) Both starch and sucrose are synthesized from trios phosphate generated by photosynthesis. Starch and sucrose synthesis are competing processes. The relationship between carbohydrates and adventitious root formation on cutting has remained controversial. The rooting capacity of many cuttings has been correlated with their carbohydrates content several researchers indicated that carbohydrates of free reducing sugars and storage carbohydrates

were important to root formation as energy and structural materials of cell to initiate root primordia (Bartolini et al. 2008, Delrio et al. 1991). These results showed that appropriate carbohydrates were good for root formation. These results are in good agreement with other study by Bartolini et al. 2008, Delrio et al. 1991, Reuveni and Raviv 1981, Dauod et al. 1989.

Plant hormones, enzymes and phenolic compounds play a significant role in internal control mechanism of rooting in olives. The endogenous phenolic compounds have different effects especially on rooting cuttings (Ercan and Ozkaya 2008, Lee et al. 2009, Perez-Bnilla et al. 2006). Future researches will be necessary in order to better understand the process of the *in vivo* rooting for the improvement of rooting protocols, by using also molecular techniques.

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