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Murat İSFENDİYAROĞLU[1]
Elmas ÖZEKER[1]

1 Ege Üniversitesi Ziraat Fakültesi,
Bahçe Bitkileri Bölümü, 35100 Bornova, İzmir,
e-posta: murat.isfendiyaroglu@ege.edu.tr

Root Regeneration of 'Domat' Olive (*Olea europaea* L.) Cuttings: Wounding Effects

'Domat' Zeytini (*Olea europaea* L.) Çeliklerinin Kök Rejenerasyonu: Yaralama Etkileri

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Key Words:

Olive, cutting, rooting, wounding.

ABSTRACT

Domat is the foremost large fruiting green table olive cultivar of Turkey. However, the root production of 'Domat' cuttings is very poor. Thus the propagation has to be done by grafting, despite it's a costly practice. In this work, the effects of five different wounding treatments on the rooting of 'Domat' olive cuttings were examined in two consecutive years. Leafy semi hardwood cuttings were collected mid-February, wounded and treated with 5 g.l^{-1} indole butyric acid (IBA) after wounding. Shallow incision wounding significantly augmented the rooting of cuttings particularly in the second year. The highest rooting percentage and root number were 68% and 4.5 respectively with incisions while unwounded cuttings gave 21% rooting and 1.0 root. Shallow slice wounding gave the longest roots (36.8 mm) in the same period. The highest number of secondary roots (2.6) was also obtained with incision wounding in the second year. Despite the highest root fresh and dry weights were obtained with no wounds in the first year, incisions gave the highest figures (276.3/30.8 mg) in the second year. Incision wounding could be the most reliable technique to improve the rooting of 'Domat' olive cuttings was concluded.

Anahtar Sözcükler:

Zeytin, çelik, köklenme, yaralama.

ÖZET

Domat' Türkiye'nin önde gelen iri yeşil sofralık zeytin çeşididir. Ancak, 'Domat' zeytini çeliklerinin köklenmesi çok yetersizdir. Bu nedenle çoğaltma, pahalı bir uygulama olmasına rağmen, aşıyla yapılmaktadır. Bu çalışmada, beş farklı yaralama uygulamasının 'Domat' zeytin çeliklerinin köklenmesi üzerine etkileri, art arda iki yıl incelenmiştir. Şubat ortasında alınan yapraklı yarı odun çeliklerine, yaralamayı takiben 5 g.l^{-1} indol butirik asit (IBA) uygulanmıştır. Yüzeysel çizme uygulaması özellikle ikinci yılda çeliklerin köklenmesini önemli düzeyde arttırmıştır. En yüksek köklenme oranı (% 68) ve kök sayısı (4.5) yüzeysel çizme uygulanan çeliklerde gözlenirken, hiç yaralama yapılmayanlarda % 21 köklenme ve 1.0 adet kök saptanmıştır. Aynı dönemde yüzeysel dilimleme yoluyla yaralanan çelikler, en uzun kök değerini (36.8 mm) vermiştir En fazla sayıda sekonder kök (2.6), yine ikinci yılda, yüzeysel çizme uygulamasıyla elde edilmiştir. En yüksek yaş ve kuru kök ağırlığı değerleri, birinci yılda hiç yaralama yapılmayan çeliklerden elde edilirken, ikinci yılda en yüksek değerleri (276.3/30.8 mg) yüzeysel çizme uygulaması vermiştir. Yüzeysel çizme şeklinde yapılan yaralamanın, 'Domat' zeytini çeliklerinin köklenmesini iyileştirmek için en güvenilir yöntem olabileceği sonucuna varılmıştır.

INTRODUCTION

The olive was probably first brought into cultivation in the Levant (Zohary and Hopf 2004), and its cultivation further developed alongside the Mediterranean civilizations. The olive is now commercially produced in the Mediterranean basin (Vossen 2007). New plantings were also made in some countries such as Argentina, Australia, Chile and South Africa where the climatic conditions are identical (Vossen 2007). Olive has been propagated from large parts (branches, shoots, ovules, suckers) since ancient times (Fabbri et al. 2004). However, propagation by leafy cuttings under mist is currently the major method in most of the olive countries (Hartmann et al. 2002; Fabbri et al. 2004). Nevertheless, some economically important olive cultivars, for example, Empeltre (Spain), Bosana (Italy) and Kalamata (Greece) known to be recalcitrant to root, so they have been propagated by grafting in practice (Barranco et al. 2000; Fabbri et al. 2004).

'Domat' is the foremost large fruiting table olive cultivar of Turkey and particularly suitable for green processing with a variety of stuffings (Barranco et al. 2000; Can and İsfendiyaroğlu 2006). So far, several investigations revealed that the rooting rate and root production of leafy 'Domat' olive cuttings are very low compared to that of better rooted cultivars (Özkaya and Çelik 1993; Gerrakakis and Özkaya 2005; İsfendiyaroğlu and Özeker 2008). Thus the nursery tree production has been done by bark grafting onto two or three years old seedling rootstocks (Can and İsfendiyaroğlu 2006). So the grafted plants are too expensive compared to own-rooted ones.

Indole butyric acid (IBA) application is useful for rooting of olive cuttings. Soaking and wounding the olive cuttings have been accepted as techniques to improve the effect of auxin treatments (Fabbri et al. 2004). Early works showed the incision wounding significantly improved the rooting of olive cultivars (Ciampi 1964; Fabbri et al. 2004). The effects of other wounding techniques in olive cuttings were unknown.

Cuttings are initially wounded when severed from stock plants. Additional basal wound is beneficial in rooting of certain woody species (Macdonald 1993; Hartmann et al. 2002). Maximum benefit from wounding is obtained by subsequently treating the cuttings with rooting hormone (Howard et al. 1984; Gonzales et al. 1989; Macdonald 1993).

Wounding induces cell division and meristematic activity of affected cells (Hartmann et al. 2002), and also increases the tissue competence to rooting hormones in cuttings (De Klerk et al. 1999). Split wounding of the hardwood cuttings of M26 apple rootstock markedly increased the rooting compared to incision and slice wounds (Howard et al., 1984). Splitting induces more potential rizogenic tissues per cutting compared to incision wounding (Mackenzie et al. 1986). Most root production from wounding was also obtained from hedges compared to stool beds in clonal apple rootstock cuttings (Howard et al. 1984).

The aim of this work is to determine the most efficient wounding technique to improve the root production of very difficult to root 'Domat' olive cuttings, and therefore to draw attention to the wounding practices in olive nursery industry.

MATERIAL AND METHODS

Semi-hardwood olive cuttings were collected on 15 February 2009 and 2010 from selected bearing mother plants growing under good care conditions at the Research Farm of the Ege University, Faculty of Agriculture, Menemen, İzmir, Turkey. One year-old vigorous water sprouts grown on the scaffold limbs of the inner tree canopy, about 60-80 cm in length and 5-7 mm in diameter were used as cutting material. Sub terminal cuttings, 20-25 cm in length, with 2-3 pairs of leaves were used. Five different wounding techniques applied to cuttings as described below:

Shallow incision wounds were made with the tip of a knife on either sides of the bark tissue to the xylem wood, for a distance of nearly 3 cm at the base of the cutting. Shallow slice wounds removed 2.5-3 cm-long slivers of tissue, minimally penetrating the xylem wood, from both sides of the proximal ends of cuttings. Deep-slice wounds were made by maximally penetrating the xylem to a depth of one-third stem thickness as opposed to the shallow slices. Split wounds were made with a knife, splitting up the center to a depth of around 2.5-3 cm at the cutting base (Hartmann et al. 2002). Finally, a second split wound was oppositely made to the first cut to make cross split wounds. Control cuttings were left unwounded.

Cuttings were soaked in a fungicide solution (0.1% prochlorase) before dipping for 5 s in 5 gl^{-1} IBA dissolved in 50% isopropanol. They were then planted in 40x20x11 cm rooting flats filled with the mixture of perlite (horticultural grade) and vermiculite (horticultural grade No.2) in equal volumes. Cuttings were rooted in a low polyethylene tunnel equipped with mist nozzles. Mist cycles were controlled by an electronic leaf during the daylight hours (1 h from dawn to 1 h before dusk in bright days). Basal heat was adjusted to 25±2ºC. A shading cloth was suspended over the tunnel and natural sunlight was initially reduced more than 90% (İsfendiyaroğlu et al. 2009). Changes in climatic parameters in low tunnel were recorded.

The design of the experiment was a completely randomized block with three replicates of 20 cuttings per treatment. Cuttings were examined ten weeks after planting to determine the rooting percentage, primary root number, root length, secondary root number, root fresh and dry weights and a visual rating (VR) score on a 0-5 scale (0: dead; 1: alive, no callus or roots; 2: little callus; 3: medium amount of callus; 4: heavily callused, and 5: rooted).

All data were subjected to analysis of variance according to completely randomized design using SPSS (version 16.0, Inc., USA) statistical software. Significant differences between means ($\alpha = 0.05$) were determined by Duncan’s multiple range tests. Results were interpreted according to the value of the probability (*P*). Mean values were given in histograms. The non-parametric VR scores were not statistically examined.

RESULTS

In the first year of the experiments, calculated mean weekly temperature and relative humidity ranged between 10.8 and 24.3°C and 85.6 and 95.3% respectively. In the second year, climatic figures were 14.3-24.5°C and 84.6-96.4% respectively.

Different cutting years did not significantly affected most of the rooting variables. Only secondary root number of cuttings was significantly ($P<0.05$) high in the second year (data not shown).

Application of different wounding techniques on olive cuttings significantly ($P<0.01$) affected most of the rooting variables except root fresh and dry weights. The incision wounding gave the highest figures in entire rooting characteristics, but techniques that needed large basal cuts (e.g. deep slice wounding, cross splitting) suppressed the root production compared to unwounded cuttings (data not shown).

The interaction cutting year x wounding technique had a significant effect on most rooting variables except for root fresh and dry weights of cuttings. In the first year, the most rooted cuttings were obtained with incision wounding (56.6%) while the unwounded cuttings gave 46.6% rooting. The rest wounding treatments more or less decreased the percent rooting of cuttings compared with unwounded ones (fig.1A). The positive effects of wounding treatments were more pronounced in the second year. Incision wounds resulted in 68.3% rooting and gave rise to more than three fold increases in percent rooting of cuttings compared with unwounded ones. Shallow slice wounding and splitting also gave 56.6 and 51.6% rooting respectively. Heavy slice wounding did markedly decrease the rooting of cuttings.

Cuttings produced most roots with incision wounds in two consecutive years. In the first year, cuttings had 3.3 roots with incisions, while they had 2.4 with no treatment. Splitting the bases of cuttings also slightly increased the root production (fig.1B). In the second year, cuttings had 4.5 roots with incision wounds compared to 1 root in untreated ones. Apart from heavy slice wounding, rest of the treatments had at least two fold increases in mean root number of olive cuttings in this period.

Splitting and incising the cutting bases slightly increased the root elongation compared to unwounded cuttings in the first year. Slice wounds and cross splitting decreased the root lengths of the cuttings rooted (fig.1C). Shallow slice wounding gave the longest roots (36.8 mm) in cuttings followed by incision (34.3 mm) and split wounding (23.5 mm) treatments in the second year. But deep slice wounding did markedly shorten the roots as opposed to other wounding treatments.

In the first year, most of the wounding treatments slightly increased the number of lateral roots compared to unwounded cuttings (fig. 1D). However, the highest number of roots (2.6) was obtained with incision wounds in the second year. The rest wounding treatments decreased the secondary root number of cuttings compared with unwounded ones.

Despite the insignificant interactions, wounding treatments markedly influenced the fresh and dry weights of roots in olive cuttings. In the first year, intact cuttings had the heaviest fresh (330.5 mg) and dry (33.1 mg) roots while the entire woundings gradually decreased the root weights as much as 134.2/12.4 mg with cross splitting for instance (fig 1E, F). But, all the woundings more or less increased the weights of roots in the second year of the rooting trials, incision wounds provided more than three fold increases in root weights (276.5/33.1 mg respectively) compared to unwounded cuttings (84.1/9.8 mg).

Evaluation of non-parametric VR scores showed none of the wounding treatments positively affected the rating scores of cuttings in the first year (fig.2A). But in the second year, all the woundings markedly increased the rating scores and consequently improved the quality of rooted cuttings (fig. 2B).

DISCUSSION

In this work, root promoting effects of different wounding treatments were more pronounced in the second year. Significant year differences in terms of root production were reported in most of the tested Turkish olive cultivars (Canözer and Özahçı 1994; İsfendiyaroğlu et al. 2009). Rooting ability of 'Domat' olive cuttings have been ascribed to alternate bearing of mature stock plants, therefore it might be related to carbohydrate status of cuttings (Özkaya and Çelik 1999). However, 'Domat' olive is known with its constant productivity (Can and İsfendiyaroğlu 2006) and moreover, non-bearing vigorous water-sprouts may not have significant year differences in carbohydrate reserves. Indirect effects of yearly climatic differences might be more determinant on rooting was thought.

Percent rooting, mean root number and root length are the most predictive variables on root quality of cuttings. Incision wounds gave the highest rooting percentage and mean root number of cuttings in two consecutive years. Also, shallow slice wound and splittings were highly efficient on root production particularly in the second year of experiments (fig. 1A, B, C). Rooting of difficult to root olive cuttings increased with incisions was previously reported (Fabbri et al. 2004). Rooting was markedly increased in parallel with the number of incisions in *Camellia* cuttings (Torres 1986), and with the depth of incision in M26 apple cuttings (Howard et al. 1984). From this point of view, increased number of incisions or deep incisions that more penetrative to the wood, might be more beneficial to improve the adventitious roots in 'Domat' olive cuttings.

Percent rooting, root number and root length were significantly high by shallow slice wounding in the second year. But deep slice wounds decreased rooting compared to controls in two consecutive years (fig. 1A, B, C). In fact, shallow slice wounds induced the rooting in cuttings of M26 apple more effectively than deep slice wounds (Howard et al. 1984). Nevertheless, it was only effective in semi-hardwood cuttings of difficult-to root *C. japonica* clone compared to the clone that had high rooting ability (Gonzales et al. 1989).

Split wounding provided significant increases in rooting variables particularly in the second year over the controls, while cross splitting was improved the percent rooting and mean root number to a lesser extent (fig. 1A, B, C). Splitting did also significantly increase the root production of hardwood apple (M26) cuttings (Howard et al., 1984). The crucial difference of split base wounding was its abundance of rhizogenic tissue per cutting compared with twin incision in apple cuttings (Mackenzie et al. 1986). In olive cuttings, expected results were not obtained, despite the two fold increase in number of rhizogenic tissue files by cross split wounding.

Despite the positive effects of most woundings on lateral root production of olive cuttings in the first year, the highest number of secondary roots was obtained with incision wounding in the second year (fig. 1D). Lateral roots are quite important for good root structure and subsequent survival of cuttings after transplanting. In relatively easy to root, unwounded 'Ayvalık' olive cuttings, production of secondary roots were reached to 13 in perlite-vermiculite 1:1 blend (İsfendiyaroğlu et al. 2009). From this point of view, poor lateral root production has to be considered as a potential drawback for further survival of 'Domat' olive cuttings.

In this work, woundings adversely affected the root weights of cuttings related to cutting year, but some techniques like cross splitting much decreased the root weights of cuttings as in the first year or little affected the weight gain as in the second year. Also, woundings like deep slicing least contributed (<10%) to dry matter gain of roots in the second cutting year (fig. 1E, F). For this reason, techniques that necessitated large wounds at the bases of cuttings might cause the depletion of some crucial substances for wound healing process rather than root development was thought.

The changes in VR scores of olive cuttings exhibited a quite similar pattern to those of root fresh and dry weights. Despite the considerable decreases in root ratings by all woundings in the first cutting year, the VR scores showed an adverse pattern in the following year of the experiment. Treatments like shallow slicing nearly doubled the rating scores compared to unwounded cuttings (fig. 2A). Rest of woundings had also high scores and thus they increased the root quality of cuttings (fig. 2B). The first year's results partially matched with the rooting of March cuttings from mature trees of 'Domat' olive had a VR score of 3.8 with 5 gl^{-1} IBA in pure perlite (İsfendiyaroğlu and Özeker 2008).

In this study, benefits of basal wounding treatments on the rooting of 'Domat' olive cuttings were proven. However, the effectiveness of wounding have seemed to be related with cutting year and therefore with the endogenous status of stock plants. Incision wounding was probably the most reliable technique to increase the number of cuttings with high root production and quality in difficult to root olives was concluded.

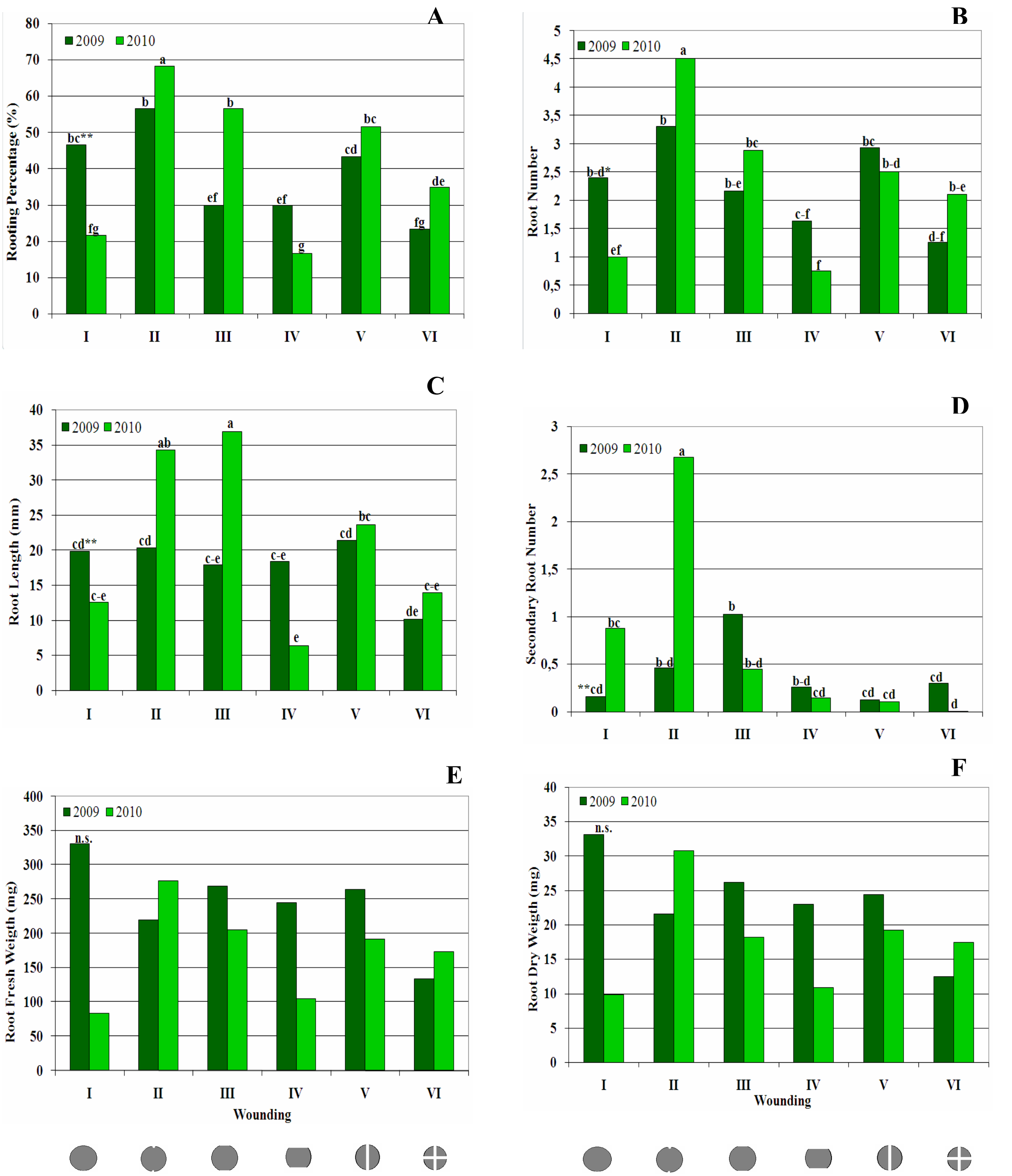

FIG. 1: The effects of different woundings on the A) rooting percentage, B) root number, C) root length, D) secondary root number, E) root fresh weight, F) root dry weight of olive cuttings. I: unwounded, II: incision, III: shallow slice, IV: deep slice, V: split, VI: cross split wounds. [a-g] Means followed by different letters are significantly different according to Duncan's Multiple Range Test. *=P<0.05; **=P<0.01 [n.s]= not significant.

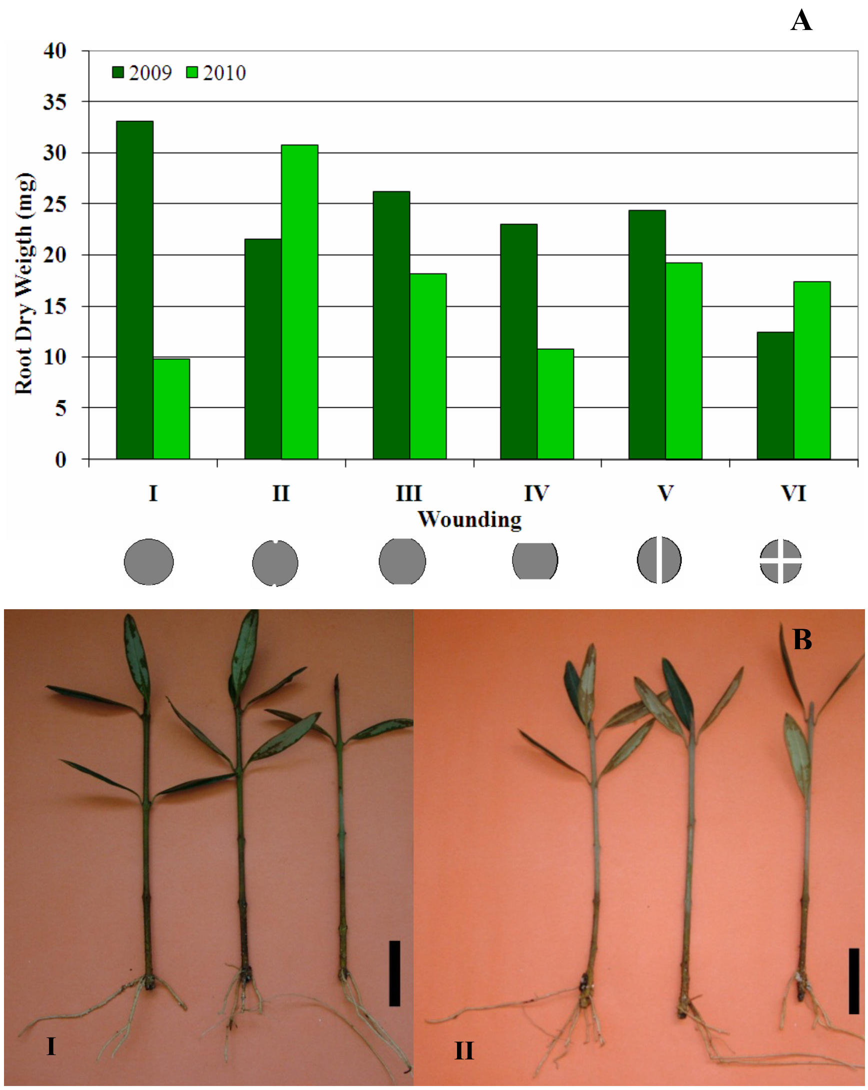

FIG. 2: The effects of different woundings on the A) visual rating, B) root structure of olive cuttings (2010). I: unwounded, II: incision wounds. Bar=5 cm.

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