

## Effects of Scion Cultivar, Rootstock Age and Hormonal Treatment on Minigrafting of Persian Walnut

Mina Farsi<sup>\*1</sup>, Mohammad Reza Fatahi Moghadam<sup>\*1</sup>, Zabihollah Zamani<sup>1</sup> and Darab Hassani<sup>2</sup>

1. Department of Horticultural Sciences, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

2. Temperate Fruits Research Center, Horticultural Science Research Institute, Agricultural Research, Education and Extension Organization, Karaj, Iran

(Received: 7 April 2018, Accepted: 9 June 2018)

### Abstract

The experiment was carried out during 2015-2016, with the aim of investigating the effects of scion cultivar, rootstock age and hormonal treatments on the success of walnut minigrafting. Analysis of results showed that all studied factors were effective on graft-take and survival of minigrafted walnuts and other growth traits of the scions. The highest graft-take was observed on one-year-old rootstocks in hormonal treatments of 20 mg/L BA + 12.5 mg/L IBA for 'Jamal' and 80 mg/L BA + 50 mg/L IBA for 'Chandler'. The highest survival rate was observed on one-year-old rootstocks in control treatment and the lowest was on two-year-old rootstocks in hormonal treatment of 80 mg/L BA + 50 mg/L IBA. The highest growth rate of scion belonged to the scions of 'Jamal' on one-year-old rootstocks in hormonal treatment of 20 mg/L BA + 12.5 mg/L IBA and the lowest was in scions of 'Chandler' on two-year-old rootstocks in hormonal treatments of 20 mg/L BA + 12.5 mg/L IBA and 80 mg/L BA + 50 mg/L IBA. In addition, diameter of the graft union on two-year-old rootstocks was more than on one-year-old ones. Histological studies showed that increase in the diameter of the graft unit on two-year-old rootstocks was due to dead and necrotic tissues at the upper parts of it which acted as a barrier against passage of water and minerals from the rootstock to the scion and reduced the growth of the scion on two-year-old rootstocks.

**Keywords:** Graft union, Graft-take, Graft survival, 'Chandler', 'Jamal', Callus.

### Introduction

Persian walnut (*Juglans regia* L.) is one of the most important nut crops with multiple difficulties for vegetative propagation (Dehghan et al., 2010; Soleimani et al., 2009). According to FAO, in 2014, Iran produced 445,829 tons of walnuts which was ranked third in the world, but due to the lack of uniformity in commodity, it could not gain a good exporting status. Use of superior cultivars and appropriate rootstocks together

with optimization of production methods are important to improve the quantity and quality of walnut production. Despite of the difficulties in vegetative propagation of walnuts, grafting is the only practical and efficient method to use the superior characteristics of the walnut cultivars (Vahdati, 2003). Budding and grafting, which are the most common methods of walnut propagation, are largely influenced by climatic conditions, especially temperature and humidity of the environment (Gandev, 2007; Karadeniz, 2005; Rezaee and Vahdati,

\* Corresponding Authors, Email: [minafarsi@ut.ac.ir](mailto:minafarsi@ut.ac.ir) and [fatahi@ut.ac.ir](mailto:fatahi@ut.ac.ir)

2008). For successful grafting of walnut, temperature around the graft union should be maintained at 27 °C following grafting (Avanzato and Atefi, 1997; Hartmann et al., 2002; Ozkan et al., 2001; Vahdati, 2006). In addition, high environmental moisture is needed for grafting because callus cells have soft walls and they lose easily their moisture when faced to dry conditions (Rezaee et al, 2008; Soleimani et al., 2010). The grafting success and survival rate of grafted walnuts are reported in several literatures, however, despite of success in the early stages, many grafted plants are failed over time (Ebrahimi et al., 2006; Gandev, 2007; Kuniyuki and Forde, 1985; Ozkan et al., 2001; Rezaee and Vahdati, 2008; Rongting and Pinghai, 1993; Solar et al., 2001). Grafting success in walnut is more difficult than other fruit trees, and requires skillful workers, appropriate rootstock and scion cultivar and proper time and method of grafting (Aminzadeh, 2012; Balanian, 2010; Dehghan et al., 2010; Gandev and Arnaudov, 2011; Ghamari Hesabi et al., 2016; Hartmann et al., 2002; Rezaee and Vahdati, 2008). In addition, phenolic compounds in walnut trees are the most important factors which reduce graft success of this plant. High concentrations of phenolic compounds lead to tissue browning after cut and prevent callus formation at the graft union (Pinghai and Rongting, 1993). Furthermore, accumulation of some phenolic compounds above the graft union affects differentiation of vascular tissues by imposing limitations on auxin transfer (Mattsson et al., 2003; Sachs, 1981). Studies have shown that in walnut minigrafting, the use of polyvinylpyrrolidone (PVP), ascorbic acid and citric acid as antioxidants reduced the oxidation of phenolic compounds and necrosis of the tissue at grafting site (Aminzadeh, 2010; Balanian, 2012).

It has been shown that plant growth regulators (such as auxins and cytokinins) have positive effects on the success enhancement grafting in walnut (Aminzadeh, 2012; Balanian, 2010; Pirkhezri et al., 2010; Zakinthinos and Rouskas, 1997). During the

early stages of grafting, increase in indole-3-acetic acid (IAA) concentration in both scion and rootstock is essential for the formation of vascular bridge between two partners (Lu et al., 1996; Lu and Yang, 1995; Zheng et al., 2010). It has been suggested that auxins are released from the vascular bundles of scion and rootstock, and play a critical role in callus induction, the cambium layer formation and vascular tissue differentiation (Aloni, 1987; Kazankaya et al., 1997; Moore, 1984; Pinghai & Rongting, 1993).

In addition, Rongting and Pinghai (1990) reported that the concentration of IAA and abscisic acid (ABA) and the IAA/ABA ratio are important factors in callus induction and vascular differentiation in walnut grafting. Furthermore, cytokinins and gibberellins can positively influence the graft success. Cytokinins are used for different purposes such as enhancement of callus formation, differentiation of the cambium layer and xylem vessels and opening of buds (Taylor, 1972; Zakinthinos and Rouskas, 1997).

The effect of 6-benzylaminopurine (BAP) as well as polyvinylpyrrolidone (PVP) and ascorbic acid antioxidants was previously investigated on graft success of walnut in different studies. Results of those investigations showed that both BAP and antioxidants have significant effects on the walnut graft-take. The best results have been obtained from treatment with 4 g/L PVP and 60 mg/L BAP (Zakinthinos and Rouskas, 1997). Alzate et al. (2002) also obtained the best results in terms of scion growth with the use of antioxidants and plant growth regulators such as BAP which caused a faster growth of the scions and allowed the grafted plants to be transported to greenhouse less than six weeks after micrografting. Pirkhezri et al. (2010) investigated the effects of auxin and gibberellin in increasing of bud break in the walnut cultivars. They reported that the best hormonal treatments in 'Chandler' were 100 ppm GA and 50 ppm IBA, while the best results in 'Pedro' and 'Z63' were achieved with 50 and 100 ppm IBA,

respectively. Aminzadeh (2012) evaluated the effect of various concentrations of IBA and BA on the minigrafting of walnut. The highest percentage of graft-take was obtained by using 50 mg/L IBA + 80 mg/L BA.

Therefore, the main objectives of the current study were to determine the effects of scion cultivar, age of rootstock and hormonal treatments on the success of minigrafting in Persian walnut.

### Materials and Methods

The experiment was conducted at the Department of Horticulture, Collage of Agriculture and Natural Resources, University of Tehran, during 2015-2016. After the fulfilment of chilling requirements in winter 2015, one- and two-year-old seedlings were transferred to greenhouse as rootstocks. In late January, scions of cvs. Chandler and Jamal were taken from one-year-old shoots of the walnut trees from experimental orchard of Kamal Shahr station of Seed and Plant Improvement Institute, Karaj, Iran. The scions were stored at cool and moist conditions, until used for grafting. In February, scions of these cultivars were minigrafted on one- and two-year-old seedlings. For minigrafting, a V-shaped cut with a length of about 0.5 to 1 cm was made at the basal end of the scions using a scalpel razor. In order to prevent the browning of the cut portion by oxidation of phenolic compounds, the scions was placed in a solution containing ascorbic acid and citric

acid (in concentrations of 500 and 1000 mg/L, respectively) for 1 min prior to the grafting. The basal end of scions was then subjected to hormonal treatments containing 0, 20, 40 and 80 mg/L BA, with 0, 12.5, 25, 50 mg/L IBA, for 1 min. After minigrafting (cleft grafting) of scions on one- and two-year-old rootstocks, grafts were wrapped with plastic strips. Then, grafted plants were covered by clear plastic cups to avoid losing of moisture (Aminzadeh, 2012; Balanian, 2010). The grafted plants maintained at  $25\pm 2$  °C temperature and 65-75% relative humidity in the greenhouse (Fig. 1 a,b).

This experiment was performed as factorial base on completely randomized block designs with two scion cultivars, two rootstock ages and four hormonal treatments. Each treatment had five replicates and each replicate included 8 grafted seedlings. In this experiment, each replication was performed in one day, and was considered as a block. Percentage of graft-take was determined four weeks after grafting and percentage of graft survival, growth rate of scions, number of nodes and the relative difference between the diameter of the graft unit and the rootstock ((diameter of the graft unit-diameter of the rootstock)/ diameter of the rootstock) were recorded four months after grafting. Statistical analyses were conducted using SAS software (SAS Institute, Cary, NC, USA), and means were compared using Duncan's new multiple range test and tables and figures were drawn with Excel software.



Fig. 1. Grafted walnuts in greenhouse covered with plastic cups. a. one day after grafting. b. forty days after grafting

## Results

Percentage of graft-take was investigated one month after minigrafting. Analysis of variance indicated that the interaction effects among scion cultivars, hormonal treatments and rootstock ages for the percentage of graft-take were significant at 5% level (Table 1).

Comparing the mean with the least significant difference (LSD) is shown in Figure 2. The highest rate of graft-take was achieved in scions of cvs. Chandler and Jamal on one-year-old rootstocks with the hormonal treatments of 80 mg/L BA + 50 mg/L IBA and 20 mg/L BA + 12.5 mg /L IBA respectively. However, they were not significantly different on one-year-old rootstocks with other hormonal combinations. They had only difference with the controls on both rootstock types but with no hormones, as well as with 'Chandler' on two-year-old rootstocks at higher hormonal combinations Main effects of hormonal treatment and rootstock age influenced the graft-take at

1% and 5% probability. Therefore, in general it seems that hormones are more effective on one-year-old rootstocks. The lowest graft-take (47.5%) was gained in scions of cv. Jamal on one-year-old rootstocks in control treatment (Fig. 2).

Percentage of graft survival was evaluated four months after minigrafting. Analysis of variance revealed that only the main effect of rootstock age and the interaction effects between hormonal treatments and rootstock ages on the percentage of graft survival were significant at 1% and 5% level respectively (Table 1). Comparing the mean of treatments by LSD test is presented in Figure 3. The highest percentage of graft survival belonged to the one-year-old rootstocks in the control treatment (96.33%), although it was not significantly different from other hormone levels (Fig. 3). The lowest survival rate was detected in control treatment (77.67%) and hormonal treatment of 80 mg/L BA + 50 mg/L IBA (71.67%) on two-year-old rootstocks (Fig. 3).

**Table 1. Analysis of variance of scion cultivars, rootstock ages and hormonal treatments on graft-take, graft survival and growth traits of minigrafted walnuts**

S.O.V	df	Mean square				Relative difference between diameter of the graft unit and the rootstock
		graft-take (%)	Graft survival (%)	Scion length	Node number	
Block	4	236.33*	436.317*	608.01 <sup>ns</sup>	0.14 <sup>ns</sup>	0.005 <sup>ns</sup>
Scion cultivar	1	48.83 <sup>ns</sup>	330.688 <sup>ns</sup>	8188.29**	3.28**	0.005 <sup>ns</sup>
Hormonal treatment	3	788.41**	124.73 <sup>ns</sup>	629.60 <sup>ns</sup>	0.87**	0.004 <sup>ns</sup>
Rootstock age	1	564.45*	3802.32**	85820.72**	0.32 <sup>ns</sup>	0.18**
Scion cultivar*	3	43.62 <sup>ns</sup>	289.6 <sup>ns</sup>	2173.41**	0.61*	0.006 <sup>ns</sup>
Hormonal treatment	1	705.08**	36.46 <sup>ns</sup>	103.97 <sup>ns</sup>	0.69 <sup>ns</sup>	0.023 <sup>ns</sup>
Scion cultivar*	3	173.83 <sup>ns</sup>	398.87*	1192.72*	0.06 <sup>ns</sup>	0.022 <sup>ns</sup>
Hormonal treatment*	3	241.54*	227.32 <sup>ns</sup>	1152.19*	0.47 <sup>ns</sup>	0.01 <sup>ns</sup>
Rootstock age	60	87.37	86.85	320.18	0.18	0.01
Error	-	14.63	12.7	13.47	7.33	35.15
C.V						

\*, \*\* = Significant at 5 % and 1%, respectively, ns= Non-significant

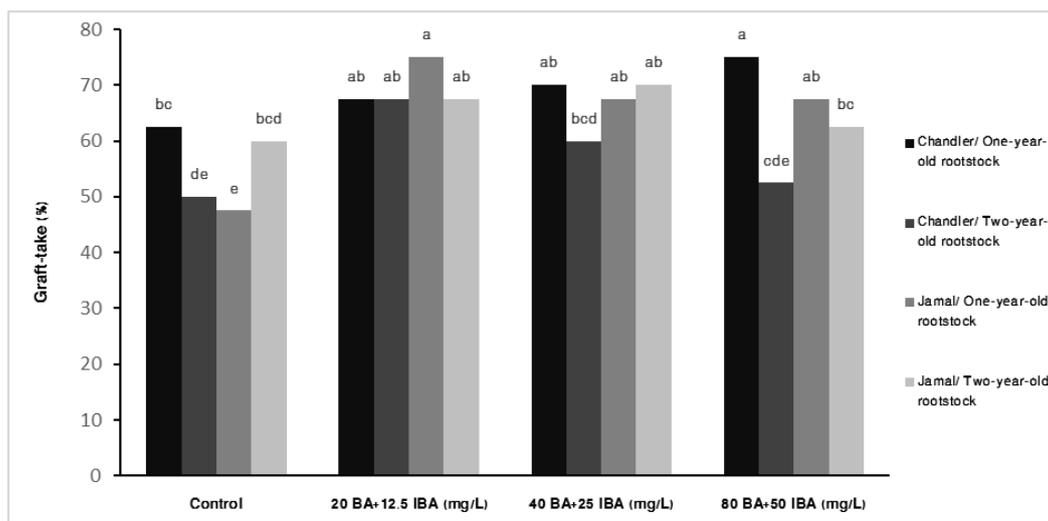


Fig. 2. Interaction effects among scion cultivars, rootstock ages and hormonal treatments for the percentage of graft-take in minigrafted walnuts

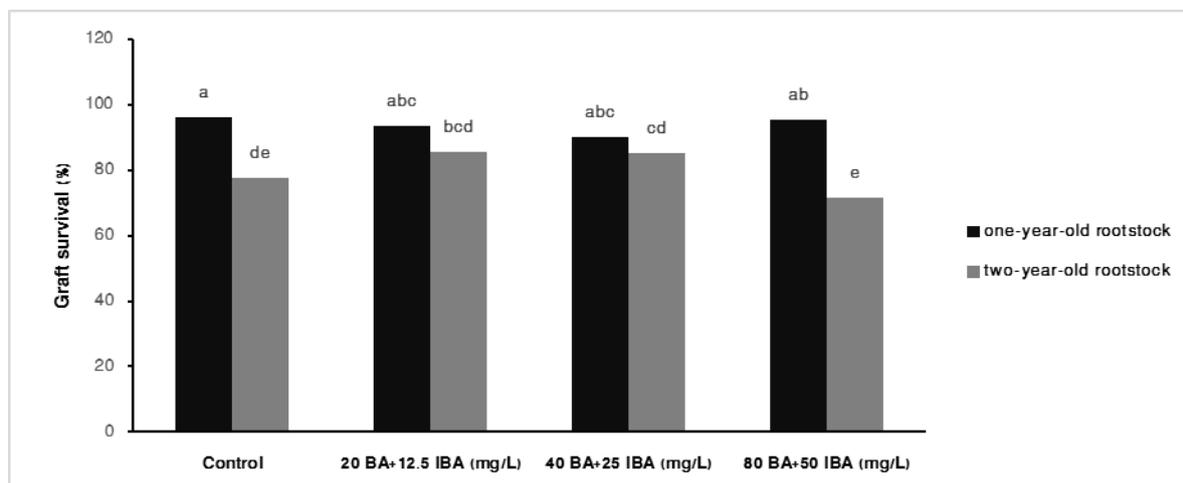


Fig. 3. Interaction effects among hormonal treatments and rootstock ages for the percentage of graft survival in minigrafted walnuts

In the present study, the length of scions was measured four months after minigrafting. Analysis of variance revealed that the effect of cultivar as well as the rootstock age on increasing the length of scions were significant at 1% level (Table 1). One-year-old rootstock increased the scion length and 'Jamal' was more elongated than 'Chandler'. Furthermore, the interaction effects among scion cultivars, hormonal treatments and rootstock ages on this trait were significant at 5% level (Table 1). Mean comparison by LSD test are presented in Figure 4. The highest length of the scions was found in

scions of 'Jamal' (196.3 mm) and 'Chandler' (178.66 mm) on one-year-old rootstocks in hormonal treatment of 20 mg/L BA + 12.5 mg/L IBA and control treatment, respectively (Fig. 4). The shortest length of the scions was observed in scions of cv. Chandler on two-year-old rootstocks in hormonal treatments of 80 mg/L BA + 50 mg/L IBA, although it was not significant with control and 80 mg/L BA + 50 mg/L IBA (Fig. 4).

Analysis of variance indicated that the effect of scion cultivar and hormonal treatment were significant at 1% level, but the interactions between scion cultivars and

hormonal treatments was significant at 5% level of probability for the node numbers (Table 1). The highest number of nodes (6.33) was in the scions of ‘Jamal’ in the hormonal treatment of 20 mg/L BA + 12.5 mg/L IBA and the lowest one (5.15) was in the scions of ‘Chandler’ in the hormonal treatment of 80 mg/L BA + 50 mg/L IBA, indicating that the highest concentration of BA and IBA hormones reduced the number of scion nodes (Fig. 5).

Eventually, the relative difference between diameter of the graft unit and the rootstock was evaluated four months after grafting. Analysis of variance revealed that the effect of the rootstock ages on the relative difference between diameter of the graft unit and the rootstock was significant at 1% level (Table 1). The relative difference of the graft site and the rootstock on two-year-old rootstocks was higher than on one-year-old ones (Fig. 6).

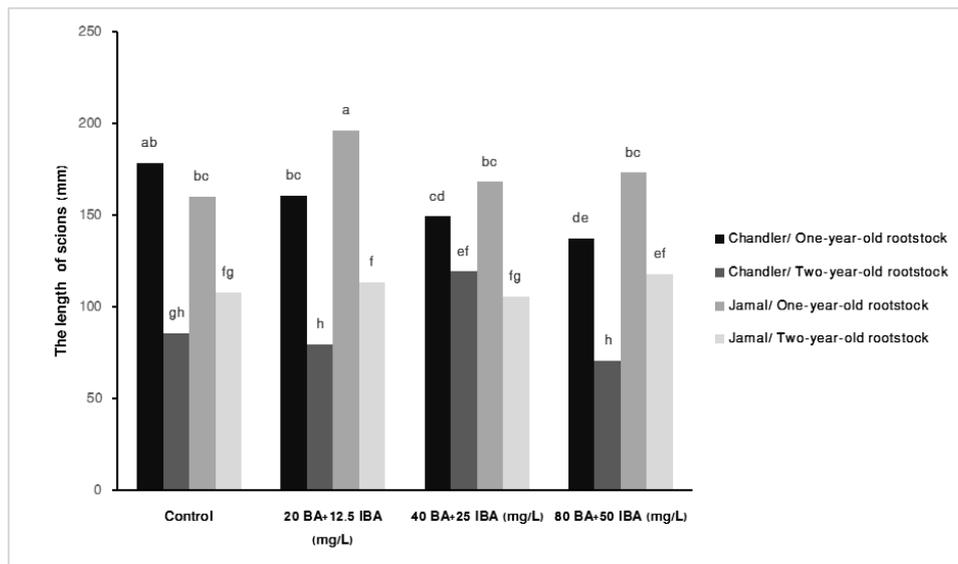


Fig. 4. Interaction effects among hormonal treatments, rootstock ages and scion cultivars for the length of scions in minigrafted walnuts

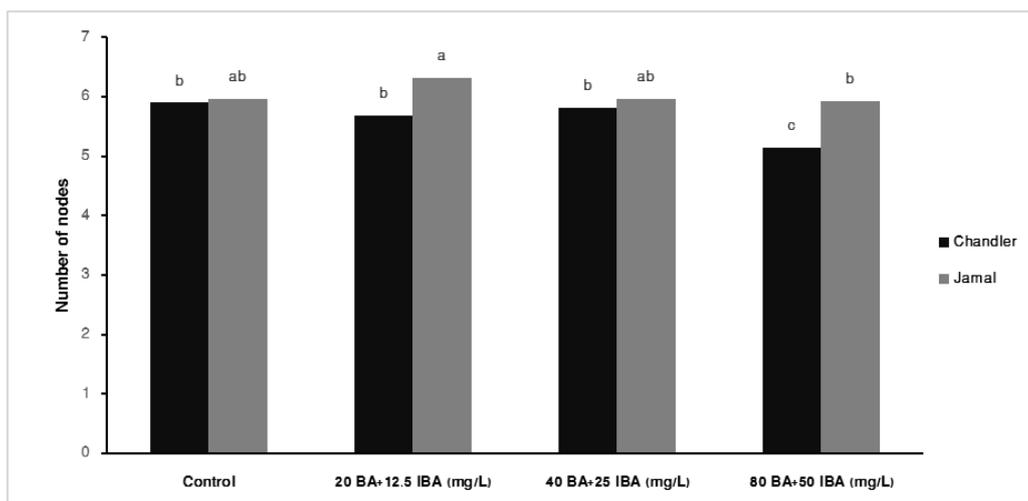


Fig. 5. Interaction effects between hormonal treatments and scion cultivars for the number of scion nodes in minigrafted walnuts

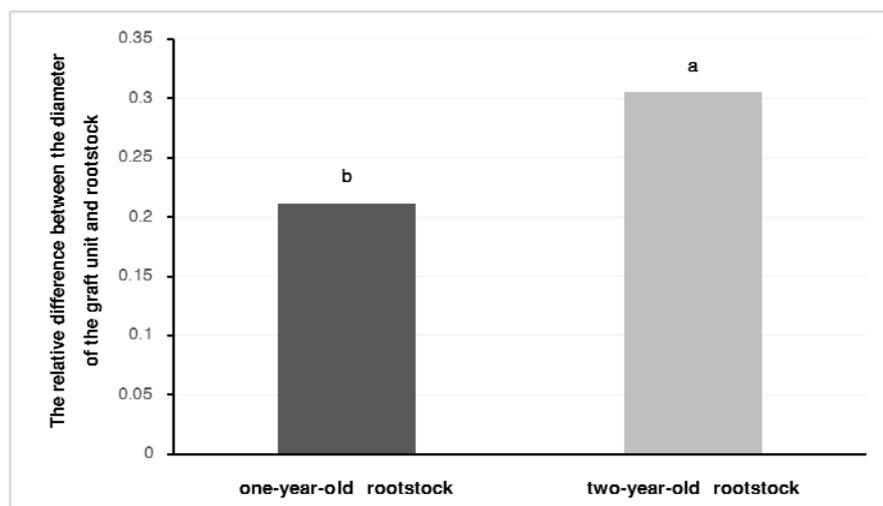


Fig. 6. Effect of rootstock age on relative difference between the diameter of the graft unit and rootstock in minigrafted walnuts

## Discussion

According to Aminzadeh (2012), the plant growth regulators of BA and IBA are effective in the success of walnut minigrafting. Researchers including Aloni et al. (2010), Hartmann et al. (2002), Mehmet et al. (1997), Rongting and Pinghai (1990) stated that hormones such as auxins and cytokinins played an important role in callus formation and vascular differentiation at the graft site. Also, Köse and Güleriyüz (2006) reported that kinetin (a type of cytokinins) and IBA promote the callus rapid proliferation between the scion and the rootstock. Aminzadeh (2012) obtained the highest percentage of graft-take on six-week-old walnuts in hormonal treatment of 80 mg/L BA + 50 mg/L IBA. In the present study, different results were obtained for the minigrafting of 'Jamal' and 'Chandler' scions between one- and two-year-old rootstocks in different hormonal treatments. The highest concentration of hormones (80 mg/L BA + 50 mg/L IBA) resulted in the highest percentage of graft-take in the scions of 'Chandler' on one-year-old rootstocks, while in the scions of 'Jamal', the highest was on one-year-old rootstocks at the lowest concentrations of hormones (20 mg/L BA + 12.5 mg/L IBA). Meanwhile, except for the control (no

hormones), no significant difference were found among concentrations of hormones on one-year-old rootstocks.

Pirkhezri et al. (2010) examined the effect of auxin and cytokinin hormones on the patch budding of walnut in Iran and stated that there was a significant difference between cultivars in response to the type of hormonal treatments. In addition, foreign cultivars showed better response to the hormonal treatments than the native genotype. They reported that the percentage of bud break in the native genotypes in response to hormonal treatments was lower than foreign cultivars which was probably due to lower levels of auxins or higher levels of auxin inhibitors.

It has been indicated that the relationship between hormones and their interactions played an important role in graft success. During the formation of callus cells, the content of IAA and the IAA/ABA ratio increased rapidly and this ratio decreased with the onset of vascular bundles differentiation. Then, during the establishment of vascular connections between two partners, IAA content and the IAA/ABA ratio increased again, and eventually, these ratios decreased after healing of the graft unit (Rongting, 1993; Rongting and Pinghai, 1990, 1993).

In the present study, the use of high levels of hormones led to decrease in graft-take on the scions of 'Chandler' on two-year-old rootstocks, but its effect was not significantly different from the control. In the scions of 'Jamal', like 'Chandler', the percentages of graft-take on one-year-old rootstocks was higher than two-year-old ones and lower concentrations of hormones (20 mg/L BA + 12.5 mg/L IBA) caused better result in term of graft take (Fig. 2).

Carlson (1963) studied the effect of different rootstock ages in grape grafting and reported that the graft site repaired more quickly on the younger rootstocks, which had more active cambium layers than the older ones. In young rootstocks, cell division was faster, and callus cells were formed more rapidly at the graft site. By comparing the minigrafting of walnut on the green and softwood rootstocks, it was found that the repair of the graft site in green rootstocks was faster than the softwood ones (Balanian, 2010); however the percentage of graft take on the softwood rootstocks (73%) was higher than green ones (40%), due to the more storage of soluble carbohydrate which provided energy to produce callus cells at the graft site. Dolgun et al. (2008) in grafting plum trees showed that when the rootstock is active, the cambium layer can produce callus cells in sufficient amount in a short time after grafting. Moreover, the water will flow properly from the root to the shoot and prevent drying of the scion. Furthermore, Rezaee et al. (2008) showed that use of highly active growing and strong seedlings of walnut as rootstock had positive effects on graft success due to their ability to absorb and transfer water and nutrients to the scion.

Successful grafting in walnut trees is a difficult process and is affected by several factors including genetic, temperature, water content and humidity, amount of soluble sugars and starch, phenolic compounds, C/N ratio in the scion and the rootstock and plant growth regulators

conditions (Hartmann et al., 2002; Pinghai and Rongting, 1993; Rongting and Pinghai, 1990; Stanisavljevic and Mitrovic, 1995). Arteca (1996) stated that plant growth regulators have a bell-shaped dose-response curve and act as inhibitors in concentrations above the maximum thresholds. In addition, Macheix et al. (1986) reported that low levels of auxins in incompatible graft units may affect the differentiation of xylem and phloem vessels and cell wall lignification. In the present study, scions of 'Jamal' on two-year-old rootstocks, similar to one-year-old ones, showed the highest percentage of graft-take at the low concentrations of hormones (20 mg/L BA + 12.5 mg/L IBA and 40 mg/L BA + 25 mg/L IBA). Moreover, similar to the scions of cv. Chandler on one- and two-year-old rootstocks, application of hormonal treatments on scions of cv. Jamal increased the graft take in comparison with the control treatment (Fig. 2).

Hartmann et al. (2002) showed that the application of IBA in the graft site led to an increase in cell division and elongation. In addition, callus cells were formed more quickly and promoted wound healing. Consequently, more complete connection with the rootstock, before losing its moisture and nutrients would be established. The type of hormones also play an important role in the success of grafting.

In the present study, there was no difference between hormone levels and control treatment for the percentage of graft survival on the one-year-old rootstocks (Fig. 3), which was probably due to the nature of rootstock which was younger and more active. On the other hand, hormonal treatments had slightly more effect on the graft survival on the two-year-old rootstocks and the lower concentrations were more effective on the graft survival on these rootstocks (Fig. 3). Various studies indicated that different hormonal concentrations had different

effects on vascular reconstruction in grafting process. It has been shown that high concentrations of IAA (1% w/w) induces differentiation of xylem vessels and lower concentrations of IAA (0.1% w/w) induces differentiation of phloem vessels (Aloni, 1987, 1995 and 2001).

In present work, the results indicated that the highest concentration of BA and IBA hormones in 'Chandler' on the one-year-old and two-year-old rootstocks had negative effects on the scion length. Similarly, the lowest concentration of hormones in the scions of 'Jamal' on the one-year-old rootstocks yielded better results.

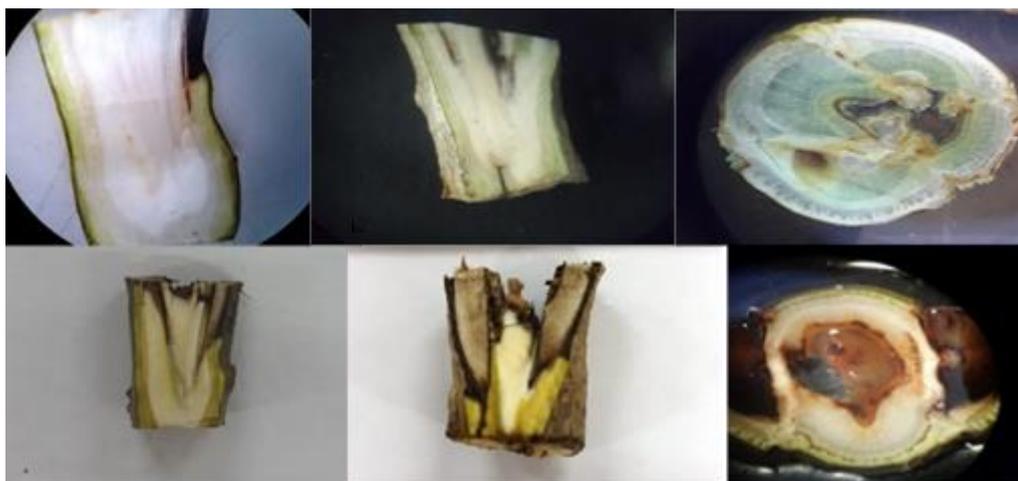
Hormonal balance is one of the main factors in the success of grafting (Aloni et al., 2008). Sorce et al. (2002) showed that in the non-grafted plants, the ratio of auxin /cytokinin was in balance, but this equilibrium was altered by grafting. Hormone imbalance between the scion and rootstock may lead to graft incompatibility (Andrews and Marquez, 1993). Studies has shown that the use of high concentrations of auxin induced ethylene synthesis, and inhibited plant growth (Mulkey et al., 1982; Rahman et al., 2001). Aloni et al. (2008) reported that use of 1-Naphthaleneacetic acid (NAA) (higher than  $10^{-10}$  M) can inhibit root growth, as the site of cytokinin synthesis. In addition, their results showed that ethylene production and  $H_2O_2$  concentration are higher in the root of the incompatible grafts than the compatible ones. It was suggested that the downward transition of auxin in incompatible grafts may reach to a concentration in root that result in inducing ethylene production. Ethylene also stimulates the production of reactive oxygen species and results in destruction of roots in incompatible graft unions. Reactive oxygen species are produced at high levels under stress conditions which are harmful for plant health due to

peroxidation of lipids, oxidation of proteins and damage of nucleic acids (Foyer and Noctor, 2003; Mittler, 2002).

In general, the percentage of graft take, graft survival and the length of scion were higher on one-year-old rootstocks which were younger and had a more active cambium layer than two-year-old rootstocks. The wound healing at the graft site on these semi-wooden rootstocks was restored in a shorter time, and vascular reconnections for transfer of water and minerals from the rootstock to the scion were more rapidly established than two-year-old wooden rootstocks. Balanian (2010) reported that highest growth of the scion and the length of the internode were obtained on younger rootstocks.

The highest number of nodes was in the scions of 'Jamal' in the lowest concentration of BA and IBA hormones. On the other hand, higher concentrations of BA and IBA hormones reduced the number of scion nodes (Fig. 5).

In present study, histological examination was carried out in the fourth month after grafting to determine the main reasons for the higher graft-take, graft survival and scion length on one-year-old rootstocks and higher relative difference between diameter of the graft unit and the rootstock on two-year-old rootstocks. The results indicated that the necrotic areas were considerably disappeared at the garft site on one-year-old rootstocks, and replaced with callus cells. Furthermore, vascular bundles were formed along the graft unit (Fig. 7 a, b and c). However, an examination of the graft union on the two-year-old rootstocks showed that upper parts of the graft site was surrounded by necrotic layers and dead cork tissues, therefore the connection between the scion and rootstock was limited to the lower parts of the graft site. The consequence of this weak connections were clearly resulted in the lower growth of the scio ns (Fig. 7 d, e, f).



**Fig. 7.** Histological observations of graft union formation in Persian walnut (*Juglans regia* L.) scions of ‘Chandler’ and ‘Jamal’ minigrafted on one- and two- year-old seedlings four months after grafting. a, b. Significant fading of necrotic layers at the graft site of ‘Chandler’ and ‘Jamal’ scions, on the one-year-old rootstocks in the longitudinal sections; c. Callus formation and vascular tissue connections at the graft site of ‘Chandler’ on one-year-old rootstock in the cross section . d, e. Presence of necrotic layers and dead cork tissues at the upper parts of the graft unit of ‘Chandler’ and ‘Jamal’ scions respectively, on two-year-old rootstocks in longitudinal sections. f. Presence of necrotic and dead tissues, low callus formation, and weak vascular connections at the upper parts of graft unit of ‘Chandler’ on two-year-old rootstock in the cross section.

Mng'omba et al. (2007) showed that accumulation of phenolic compounds and necrotic tissues at the upper parts of the graft unit can barricade the continuity of the vascular tissue connections and act as a barrier against transfer of water and nutrients from the root to the shoot, resulting in poor growth of the scion in *Uapaca kirkiana* plant.

The excessive increase in the diameter of the graft site can act as a factor for graft incompatibility. In grafted apricots, Tshokoeva and Tsonev (1995) observed a significant increase in the diameter of incompatible graft sites. Mng'omba et al. (2007) also pointed out that the growth of the graft site was significant in incompatible grafts of *Uapaca kirkiana*, creating transverse gaps in the skin. They also showed that increase in the diameter of graft site is associated with accumulation of metabolites such as phenolic compounds and carbohydrates.

Errea (1998) stated that high levels of callus tissues forming parenchymal cells result in swelling of the graft area. Furthermore, the transmission problems

due to accumulation of some substances can lead to swelling in this area. In the present work, histological studies indicated that presence of necrotic and dead tissues in the site of grafting on the two-year-old rootstocks, caused swelling of the tissue at this area, and acted as a barrier against the passage of water and nutrients from the rootstock to the scion.

## Conclusions

Present study showed that scion cultivar, rootstock age, different concentrations of BA and IBA hormones and their interactions were involved in the graft-take, graft survival and other growth traits in walnut grafting. Minigrafting on one-year-old rootstocks was better than on the two-year-old ones. In addition, hormonal treatments that increased graft-take and graft survival were not the same for the scions and rootstocks. It is suggested that in future studies, effects of different cultivars and genotypes and effects of different hormones should be taken into account for walnut grafting. Furthermore, change of phenolic compounds should be

studied in order to provide a more clear view of the factors affecting the grafting success in walnut.

## References

- Aloni B, Karni L, Deventurero G, Levin Z, Cohen R, Katzir N, Lotan-Pompan M, Edelstein M, Aktas H, Turhan E, Joel D.M, Horev C, Kapulnik Y. 2008. Physiological and biochemical changes at the rootstock-scion interface in graft combinations between *Cucurbita* rootstocks and a melon scion. The Journal of Horticultural Science and Biotechnology 83, 777-783.
- Aloni R. 1987. Differentiation of vascular tissues. Annual Reviews Plant Physiology 38, 179-204.
- Aloni R. 1995. The induction of vascular tissues by auxin and cytokinin. In: Davies P.J. (Ed.) Plant Hormones: Physiology, Biochemistry and Molecular Biology. Kluwer Academic Publishers, Dordrecht, The Netherlands. pp 531-546.
- Aloni R. 2001. Foliar and axial aspects of vascular differentiation hypothesis and evidence. Journal of Plant Growth Regulation 20, 22-24.
- Aloni R. 2010. The induction of vascular tissues by auxin plant hormones. In: Davies P.J. (Ed.) Plant Hormones: Biosynthesis, Signal Transduction and Action. Kluwer Academic Publishers, Dordrecht, The Netherlands. pp 485-518.
- Alzate A, Royero N, Nunez V, Cabral J, Tohme J, Mejia-Jimenez A. 2002. Optimization of the *in vitro* propagation methodology of selected clones of soursop (*Annona muricata* L.) and evaluation of the compatibility of different scion and rootstock combinations for *in vitro* micrografting. Centro Internacional de Agricultura Tropical-CIAT, Cali, CO.
- Aminzadeh F. 2012. An Investigation on Minigrafting of Walnut in Semi-Controlled Conditions. University of Tehran, Iran. M.Sc. Thesis. P 113.
- Andrews P.K, Marquez C.S. 1993. Graft incompatibility. Horticultural Reviews 15, 183-232.
- Arteca R.N. 1996. Plant Growth Substances: Principles and Applications. Chapman & Hall, New York. P 332.
- Avanzato D, Atefi J. 1997. Walnut grafting by heating the graft point directly in the field. Acta Horticulturae 442, 291-294.
- Balanian H. 2010. Walnut Minigrafting in Controlled Conditions. University of Tehran, Iran. M.Sc. Thesis. P 110.
- Carlson V. 1963. How to green graft grapes. Univ. of California, USA. P 115.
- Dehghan B, Vahdati K, Hassani D, Rezaee R. 2010. Bench-grafting of Persian walnut as affected by pre- and post-grafting heating and chilling treatments. The Journal of Horticultural Science and Biotechnology 85(1), 48-52.
- Dolgun O, Tekintas F.E, Ertan E. 2008. A histological investigation of graft union in some plum varieties grafted on pixy rootstock. ADu Ziraat Fakultesi Dergisi 5, 1-4.
- Ebrahimi A, Vahdati K, Fallahi E. 2006. Improved success of Persian walnut grafting under environmentally controlled conditions. International Journal of Fruit Science 6, 3-12.
- Errea P. 1998. Implications of phenolic compounds in graft incompatibility in fruit tree species. Scientia Horticulturae 74, 195-205.
- FAO. 2014. FAOSTAT database results. <http://faostat3.Fao.org/faostat>.
- Foyer C.H, Noctor G. 2003. Redox sensing and signaling associated in plants: A reevaluation of the concept of oxidative stress response in leaves. Journal of Experimental Botany 53, 1249-1254.
- Gandev S. 2007. Budding and grafting of the walnut (*Juglans regia* L.) and their effectiveness in Bulgaria (Review). Bulgarian Journal of Agricultural Science 13, 683-689.
- Gandev S, Arnaudov V. 2011. Propagation method of epicotyl grafting in walnut (*Juglans regia* L.) under production condition. Bulgarian Journal of Agricultural Science 17, 173-176.
- Ghamari Hesabi F, Sharafi Y, Tabatabaei S.J, Grigurian V. 2016. Effect of budding method, rootstock age and cut below budding union on budding success in Persian walnut. Journal of Nuts 7(2), 119-124.
- Gross G.G, Janse C, Elstner E.F. 1977. Involvement of malate, monophenols, and the superoxide radical in hydrogen peroxide formation by isolated cell walls from horseradish (*Amoracia lapathifolia* Gilib.). Planta 136, 271-276
- Hartmann H.T, Kester D.E, Davies F.T.J.R, Geneve L.R. 2002. Plant Propagation: Principles and Practices. Seventh Edition. Regents / Prentice Hall International Editions, Englewood Cliffs, New Jersey. P 880.

24. Karadeniz T. 2005. Relationships between graft success and climatic values in walnut (*Juglans regia*). Journal of Central European Agriculture 6, 631-634.
25. Kazankaya A, Mehmet S, Tekintas F.G. 1997. Relations between graft success and structural hormones on walnut (*J. regia* L.). Acta Horticulturae 442, 295-298.
26. Köse C, Güteryüz M. 2006. Effects of auxins and cytokinins on graft union of grapevine (*Vitis vinifera*). New Zealand Journal of Crop and Horticultural Science 34(2), 145-150.
27. Kuniyuki A, Forde H. 1985. Walnut propagation. In: Ramos D. (Ed.). Walnut Orchard Management. Publication 21410, University of California, USA. pp 38-46.
28. Lu S.F, Yang S.J. 1995. Electrical wave transmission between stock and scion. Acta Phytophysiological Sinica 21, 386-392.
29. Lu S.F, Tang D.T, Song J.Y, Liu M.Q, Yang S.J. 1996. Preliminary studies on controlling graft union through plant hormones. Acta Botanica Sinica 38, 307-311.
30. Macheix J.J, Fleuriet A, Quessada M.P. 1986. Involvement of phenols and peroxidases in wound healing and grafting. In: Greppin H, Penel C, Gaspar T.H (Eds). Molecular and Physiological Aspects of Plant Peroxidases. Univ. of Geneva, Switzerland. P 267.
31. Manusev B. 1970. Studies on some method of grafting walnuts in the region of Force. Horticulture Abstract 41, 6040.
32. Mattsson J, Ckurshumova W, Berlet T. 2003. Auxin signaling in *Arabidopsis* leaf vascular development. Plant Physiology 131, 1327-1339.
33. Mehmet S, Kazankaya A, Testereci H, Hakkıyörük I. 1997. Changing of IAA (Indol-3-Acetic Acid) content at different organs of walnut (*Juglans regia* L.), after grafting. Acta Horticulturae 422, 169-174.
34. Mittler R. 2002. Oxidative stress, antioxidants and stress tolerance. Trends in Plant Sciences 7, 405-410.
35. Mng'omba S.A, Du Toit E.S, Akinnifesi F.K, Venter H.M. 2007. Histological evaluation of early graft compatibility in *Uapaca kirkiana* Müell Arg. scion/stock combinations. HortScience 42(3), 1-5.
36. Moore R. 1984. A model for compatibility-incompatibility in higher plants. American Journal of Botany 71(5), 752-758.
37. Mulkey T.J, Kuzmanoff K.M, Evan M.L. 1982. Promotion of growth and shift in the auxin dose/response relationship in maize roots treated with the ethylene biosynthesis inhibitors aminoethoxyvinylglycine and cobalt. Plant Science Letters 25, 43-48.
38. Ozkan Y, Edizer Y, Akca Y. 2001. A study on propagation with patch budding of some walnut cultivars (*Juglans regia* L.). Acta Horticulturae 544, 521-525.
39. Pinghai D, Rongting X. 1993. Effect of phenols on survival of walnut grafting. Acta Horticulturae 311, 134-140.
40. Pirkhezri M, Hasani D, Soleimani A. 2010. Effects of IBA and GA3 on increasing the efficiency of patch budding in some Persian walnut cultivars. Horticultural Science 24(1), 64-69. (In Persian)
41. Prativiera A.G, Kuniyank A.H, Ryogo K. 1983. Growth inhibitors in xylem exudates of Persian walnut and their possible role in graft failure. Journal of the American Society for Horticultural Science 108, 1043-1045.
42. Rahman A, Amakawa T, Goto N, Tsurumi S. 2001. Auxin is a positive regulator of ethylene-mediated response in the growth of *Arabidopsis* roots. Plant and Cell Physiology 42, 301-307.
43. Rezaee R, Vahdati K. 2008. Introducing a simple and efficient procedure for top working Persian walnut trees. Journal of the American Pomological Society 62, 21-26.
44. Rezaee R, Vahdati K, Grigoorian V, Lizade M. 2008. Walnut grafting success and bleeding rate as affected by different grafting methods and seedling vigor. The Journal of Horticultural Science and Biotechnology 83, 94-99.
45. Rongting X, Pinghai D. 1990. Theory and practice of walnut grafting. Acta Horticulturae 284, 69-89.
46. Rongting X, Pinghai D. 1993. A study on the uniting process of walnut grafting and the factors affecting. Acta Horticulturae 311, 160-170.
47. Rongting X. 1993. A study on the uniting process of walnut grafting and the factors affecting. Acta Horticulturae 311, 160-172.
48. Sachs T. 1981. The control of the patterned differentiation of vascular tissue. Advances in Botanical Research 9, 151-262.
49. Solar A, Stampar F, Trost M, Barbo J, Avsec S. 2001. Comparison of different propagation methods in walnut (*Juglans regia* L.) made in Slovenia. Acta Horticulturae 544, 527-530.

50. Soleimani A, Rabiei V, Hassani D. 2010. Effect of different techniques on walnut (*J. regia* L.) grafting. *Journal of Food, Agriculture and Environment* 8(2), 544-546.
51. Soleimani A, Rabiei V, Hassani D, Amiri M.E. 2009. Effects of rootstock and cultivar on propagation of Persian walnut (*Juglans regia* L.) using hypocotyle grafting. *Seed & Plant Production* 25(1), 93-101. (In Persian)
52. Sorce C, Massai R, Picciarelli P, Lorenzi R. 2002. Hormonal relationships in xylem sap of grafted and ungrafted *Prunus* rootstocks. *Scientia Horticulturae* 93, 333-342.
53. Stanisavljevic M, Mitrovic M. 1995. Effect of variety on success grafting and development of nursery trees of walnut (*J. regia*). *Acta Horticulturae* 442, 281-283.
54. Taylor R.M. 1972. Influence of gibberellic acid on early patch budding of pecan seedling. *Journal of the American Society for Horticultural Science* 97(5), 677-679.
55. Tshokoeva M.D, Tsonev R.V. 1995. Compatibility of rootstock and scions in apricots trees. *Acta Horticulturae* 384, 471-476.
56. Vahdati K. 2003. Nursery management and grafting of walnut. Khaniran Publication, Tehran, Iran. P 99. (In Persian)
57. Vahdati K. 2006. Evaluation of side-stub and hypocotyl grafting efficiency for walnut propagation in Iran. *Acta Horticulturae* 705, 347-351.
58. Zakinthinos G, Rouskas D. 1997. Specific treatment on walnut grafting improvement. *Acta Horticulturae* 442, 285-289.
59. Zheng B.S, Chu H.L, Jin S.H, Huang Y.J, Wang Z.J, Chen M, Huang J.Q. 2010. cDNA-AFLP analysis of gene expression in hickory (*Carya cathayensis*) during graft process. *Tree Physiology* 30, 297-303.