Development and extension of walnut propagation in Iran

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Abstract
Persian walnut (*Juglans regia* L.) is widely grown in Iran, as the center of origin of this tree. Propagation of this species is very difficult compared to other fruit and nut trees. Different types of grafting (epicotyl grafting, side stub grafting, omega grafting, whip and tongue grafting, saddle or V grafting, patch budding, chip budding, shield budding and topworking) and tissue culture (propagation by micro shoots or somatic embryogenesis) techniques have been studied intensively in the last 25 years in Iran. In these studies, effect of grafting type and time under different conditions (greenhouse, outdoor and shade house) on callus quality, graft-take, survival and growth of the scions have been studied. Several experiments have been conducted on optimization of micro-propagation steps such as establishment, proliferation, rooting and acclimatization of walnut. Successful techniques have been taught to the growers and agricultural experts. As a result of these efforts, several walnut grafting nurseries and tissue culture labs have been established which propagate walnut cultivars and rootstocks commercially for local and international markets.

Keywords: *Juglans regia*, grafting, budding, micropropagation, topworking

INTRODUCTION

The origin of common walnut (*Juglans regia* L.) is found in Persia about 2000 BC (Cociu et al., 2007). Persian walnuts (*J. regia* L.) are widely grown in Asia and Iran (Vahdati et al., 2004). Iran was ranked as the second largest walnut producer in 2012 by producing 450,000 t (FAO, 2015). The previous studies demonstrated that sexual propagation methods of walnut had undesirable results. Persian walnut is propagated by seedlings in several countries including Iran. Vegetative propagation in walnut is more difficult in comparison with other fruit and nut trees (Ozkan and Gumus, 2001). Walnut vegetative propagation by cuttings is very difficult due to their low rooting ability.

GRAFTING AND BUDDING OF WALNUT

Different methods of walnut propagation are being investigated all around the world and one of the best methods to propagate Persian walnut is the use of graft techniques. Walnut grafting success was reported to be affected by graft techniques (Mitrovic, 1995). Graft technique types including epicotyl grafting, side stub grafting, omega grafting, whip and tongue grafting, saddle or V grafting, patch budding, chip budding, shield budding and topworking and these techniques were examined by various researchers (Achim and Botu, 2001; Avanzato, 2001). However, there is not a common opinion worldwide on the choice of a certain grafting method. Grafting success in walnut is affected by multiple factors including time and method of grafting (Solar et al., 2001; Tshering et al., 2006), temperature and humidity (Barut, 2001; Avanzato, 2009), phenolic compounds, hormonal condition, choice of cultivars and rootstocks (Mitrovic et al., 2008), and time of taking the scions (Paunovic et al., 2012).

Bench grafting is one of the classic techniques for propagation or topworking of Persian walnuts (*J. regia*). Scientific literature reports different data on the efficiency of these methods in different countries. The most popular bench grafting methods include cleft,

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whip, omega, saddle and side grafting. The previous studies showed that different bench grafting methods had significant different effects on callus quality, graft take and grafting survival. In a study in Iran, the results demonstrated that omega method had best grafting success among all combinations in four walnut cultivars ('Hartley', 'Pedro', 'Serr' and 'Z53') (Dehghan et al., 2009). In this study, omega grafting showed the highest callus quality (2.5 from 4), high graft take (67.77%) and grafting survival (84.33%) as well as high scion growth (12.9 cm) followed by side stub and whip grafting. As demonstrated in this study, percentage of graft take shows a highly positive correlation with callus quality at different grafting methods. The same researchers in another study showed that omega grafting gave the highest callus rating (scoring 2.6 out of 4.0), the greatest number of callused plants (82%), the most graft-take (71%), and the highest graft survival rate (81%) in comparison with whip and side grafting (Dehghan et al., 2010). In another study, the effect of grafting type (clef, whip and bark grafting) on grafting success was performed (Rezaee et al., 2008). In this study, the percent of graft take, three weeks after grafting, for both grafting (clef and whip grafting) methods used in the preliminary trial was 80%. However, many scions failed to continue to grow and did not survive one year after grafting. Percentages of grafting survival were about 37% in clef and 0% in whip tongue grafting. This failure may be a result of high root pressure of walnut trees in early spring or winter frost damage due to weak or too fast growth of scions. In contrast, the modified bark grafting performed at mid-April showed the highest grafting success (100%) and survival percentage (96.3%). During the recent years, hypocotyl grafting technique was introduced in practice (Vahdati and Zareie, 2006; Gandev and Dzhuvinov, 2006). In this method, grafting takes place during vegetation with growing tip of soft wood cutting on clef. In the same line, the result showed that side-stub was better than hypocotyl grafts and grafting success in side-stub and hypocotyl grafts was 98 and 70%, respectively (Vahdati and Zareie, 2006).

In the same field, Soleimani et al. (2010) evaluated the effect of two grafting methods (omega and saddle) with two hot callusing systems including hot callusing in room (HCR) and modified hot callusing cable (MHCC) on grafting success and loss. The results demonstrated that usage of MHCC system resulted in the highest graft success (63.3%) and lower graft loss (28.1%) comparing with HCR system with 56.7% success and 36.4% loss. Based on the results, Soleimani et al. (2010) announced that graft success was higher using saddle method (87.7%) than omega method (38.3%). Also, graft loss was lower with saddle method than omega method (19 and 45.5%, respectively).

Suk-In et al. (2006) reported a new and rapid approach for walnut propagation called epicotyl grafting. They demonstrated that the survival rate was 78.9% in average when applying that technique. In the same line, a study carried out to evaluate the effects of scion properties on success of walnut epicotyl grafting and early growth of the grafted trees in Iran (Raofi and Vahdati, unpublished). Authors stated that scions obtained from apex and including two buds, with 3-6 mm diameter and 7-12 cm length produced a better callus, more grafting success and survival rate, and higher shoot length and leaf number tree⁻¹.

Budding is one of the popular techniques for Persian walnuts propagation in Iran. The most important budding methods are patch, I-shield and chip budding. Patch budding is one of the oldest and popular techniques for propagation in a nursery in outdoor condition (Kuniyuki and Forde, 1985). In a study in Nieriz, Fars province, Iran, Ebrahimi et al. (2007) stated that the highest success rate was obtained with patch (91.0%) followed by shield (31.1%) and chip (19.1%) budding. Patch budding showed the highest callus formation and scion growth followed by shield and chip budding. Results obtained in this study demonstrated that percentages of "bud-take" were affected by different methods of budding under greenhouse and field conditions. Other studies were conducted to determine the best method for improving the quality and productivity of walnut trees through topworking during 2001-2006, in Kahriz Agricultural Research Station located in the northwest of Iran (Rezaee et al., 2014). In this study, patch, and I-shaped budding was done after bark slipping (April 16-20) but chip budding was completed before bark slipping (March 25 and 26) using shoots with 1.5 to 2 cm in diameter. The percent of graft take, three weeks after grafting, for all of the budding methods (chip, patch and I-shaped) used in the preliminary trial was very
high (73 to 80%) but these methods produced no scion survival. Pirkhezri et al. (2010) evaluated the effects of Indole-3-butyric acid (IBA) and gibberellic acid (GA3) on increasing the efficiency of patch budding in four cultivars and one genotype of Persian walnut. The results showed that ‘Chandler’ had the highest response to GA3 100 ppm and IBA 50 ppm, ‘Pedro’ to IBA 50 ppm, while in ‘Z63’, IBA 100 ppm had the best effect. Based on these results, it was assumed that suitable hormone treatment could overcome a part of problems relating to low level of graft success in Persian walnut.

Time of grafting is one of the important factors in grafting success of Persian walnut. Gandev and Arnaudov (2011) showed that time of grafting influenced the success of propagation and epicotyl grafting method. Suk-In et al. (2006) reported that the survival rate ranges from 65.0 to 85.5% depending on the time of propagation. In the same field, a study by Rezaee et al. (2014) was performed to determine the most suitable method and time for topworking of the inferior mature walnut trees in northwest of Iran. The results showed that the best time (in Kahriz Agricultural Research Station located in northwest of Iran) for modified bark grafting was mid-April, a few days after bud burst. In another study, Aminzadeh et al. (2013) stated that time of grafting has significant effect on graft take and grafting survival and they determined that the most suitable time for minibudding is February with 83.24% grafting success compared to grafting performed at other times (early January, March and early May). Ebadi and Solagi (2002) stated that the best time for side and saddle grafting in Persian walnut is late December to late January. Efficiency of two methods of budding including patch and chip budding in three times (September, March and April) were tested by Vahdati et al. (unpublished). The results of this study showed that maximum success for patch budding and chip budding were recorded in the late April (68.75%) and late September (55%), respectively.

Kind of graft union coverage can be another important factor for walnut propagation in outdoor condition. In the same way, a study was carried out to understand the effect of kind of graft union coverage (white polyethylene tape, bicycle tube, plaster (adhesive tape), sawdust, plaster + sawdust) on grafting success. According to the results, the highest rate of callusing (4.6 from 5), grafting success (65%), and scion growth (91.25 cm) was obtained through covering of graft union with plaster + sawdust (Rezaee and Vahdati, unpublished). Another study was performed to understand the effect of kind of graft union coverage (cocopeat + soil + manure and sawdust) on graft take success. The results revealed that covering by sawdust was better than mixed coverage (cocopeat + soil + manure) and control in terms of callus formation, graft-take, survival and growth of the scion (Sadeghi Majd and Vahdati, unpublished).

Rootstock and scion genotype significantly influenced the percentage of successful bench grafting and budding. Genotypes/cultivars showed differential response to graft take success (Ferhatoglu, 1997; Erdogan, 2005). Studies conducted in Iran have confirmed this entry (Ebrahimi et al., 2007; Soleimani et al., 2009; Dehghan et al., 2009; Pirkhezri et al., 2010). For example, Soleimani et al. (2009) marked a clear effect of rootstock and scion genotype on propagation of Persian walnut and the graft take was also significantly different among the cultivars. The results of this study showed that graft take was higher on offspring of ‘Serr’ (65.8%) in comparison with ‘Z63’ (46.7%). On the other hand, there was an interaction effect considering both factors (rootstocks and scions) so that all cultivars except ‘Z63’ showed better results on ‘Serr’ rootstocks.

MICROPROPAGATION OF WALNUT

Micropropagation is the best method for propagation of all plants in a large scale. Micropropagation can be used for large-scale multiplication of cultivars with desirable traits and the production of healthy and disease-free plants. The micropropagation of J. regia is possible via the cultivation of nodal segments, cotyledon, immature cotyledons, ovules, mature embryos and meristems (Aly et al., 1992; Pijut, 1993a, b; Long et al., 1995; Vahdati et al., 2004; Aviles et al., 2009). Progress in developing methods for micro-propagation of J. regia has been very successful over the past decade. Walnut micropropagation is one of the methods studied for propagation of walnut genotypes with desirable traits in Iran. A study
was carried out to root and acclimatize in vitro grown shoots from mature walnut trees. Rooting was successfully done in Persian walnut cultivars (Figure 1). Data from this study showed that there is a positive relationship between the vigor of cultivars and rooting ability (Vahdati et al., 2004). Vahdati et al. (2009) studied the micropropagation of some dwarf walnut genotypes. The results revealed that number of axillary shoots rising from the microshoots was the highest in dwarf and semi-dwarf genotypes compared to the high vigorous ones.

Figure 1. Successful rooting of walnut by micropropagation.

Plantlets grown in conventional tissue culture systems usually encounter physiological and anatomical abnormalities. Forced ventilation can improve the development of leaf wax as well as the survival of the plants in vitro culture (Zobayed et al., 2001). In the same way, Hassankhah et al. (2014) reported that natural ventilation had a significant effect on most of the growth indices in Persian walnut.

Sucrose is frequently used as a carbon source at a concentration of 2-5% in plant culture media. The importance of sucrose in micropropagation as a carbon source has been well shown (Hazarika et al., 2004). It was reported that usage of sucrose with 15 g L⁻¹ produced healthy plantlets (Hassankhah et al., 2014). Also, Vahdati et al. (2004) showed that increasing the sucrose level in the root induction medium improved rooting.

Medium mineral composition has been reported to affect morphogenic responses. In another study, two modified media (mDKW) were formulated based on the walnut explant mineral contents and growth rate of explants cultured on mDKW was compared to those cultured on DKW medium. In this study it was found that no deficiency symptoms were observed in explants on mDKW media. Also, explants grown on mDKW produced the maximum stem length and auxiliary bud number (Najafian Ashrafi et al., 2010). The same authors conducted a study to evaluate the effects of Cu and myo-inositol on shoot length and rooting rate of explants. The results of this study showed that the higher levels of Cu and myo-inositol would be affective on growth rate and rooting percentage of walnut explants (Najafian Ashrafi et al., 2009). A study was performed to evaluate the effects of two chelates (FeEDDHA and FeNa₂EDTA) with four concentration of iron (45, 91, 182 and 360 mg L⁻¹) on growth and rooting. Results showed that the best treatment was 182 mg L⁻¹ iron with EDDHA chelate. Increasing iron concentration to 182 mg L⁻¹, caused better growth and rooting, but increasing to 360 mg L⁻¹ decreased these factors. About of FeNa₂EDTA chelate, growth and rooting was lower than same concentration in EDDHA chelate (Najafian Ashrafi and Vahdati, unpublished). Micropropagation of walnut has been commercialized in Iran and thousands of plants are produced and delivered by this method (Figure 2). Commercial modern orchards are being increased using own-rooted walnut cultivars under consultation of the experts in Iran (Figure 3).
Figure 2. Commercial mass propagation of walnut by tissue culture in Iran.

Figure 3. Establishment of a new modern orchard by own-rooted tissue culture plants of walnut in Iran.

About somatic embryogenesis, Vahdati et al. (2008) studied the effects of abscisic acid (ABA), on the maturation and germination of Persian walnut somatic embryos. The results showed that the best treatment for germination contained 2 mg L⁻¹ ABA and resulted in 41% conversion of embryos into plantlets. On the other hand, the importance of sucrose on increase of the number of secondary embryos has been documented by Vahdati et al. (2008).

CONCLUSIONS

Undoubtedly, the results of research have markedly improved traditional walnut propagation techniques such as budding, grafting and micropropagation in Iran, during the last decade. Based on the research results on budding, we recommend producing grafted walnut plants using patch budding in right place and time of the year and/or under greenhouse conditions. Among grafting techniques, whip and tong and saddle grafting using right wrapping methods and also hot callus systems are also promising. The clonal propagation of walnut via rooting of microcuttings from original plants is very useful not only for mass rapid propagation but also for maintaining valuable walnut rootstocks and genotypes. Walnut micropropagation techniques have been commercialized in Iran and tissue culture plants and know-how for producing them are available to export and transfer to the other countries.
Literature cited


