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Persian walnut (*Juglans regia* L.) grafting as influenced by different bench grafting methods and scion cultivars

Babak Dehghan[1], Kourosh Vahdati[1]*, Reza Rezaee[2] and Darab Hassani[3]

*[1]Department of Horticulture, College of Abouraihan, University of Tehran, Tehran, Iran, [2]Agricultural and Natural Resources Research Center, West Azerbaijan, Uromia, Iran, [3]Department of Horticulture, Seed and Plant Improvement Institute (SPII), Karaj, Iran. *E-mail: kvahdati@ut.ac.ir*

Abstract

The study was conducted to determine the effects of different grafting methods and scion cultivars on walnut grafting under controlled conditions in March 2006. Four walnut cultivars ('Z53', 'Hartley', 'Pedro' and 'Serr') were grafted using three bench grafting methods (side stub, omega and whip tongue) onto dormant two years old Persian walnut seedlings as rootstock. The plants after grafting were covered with moist sawdust with relative humidity of 85- 90% and stored in a humid room at 26-28 ºC for 21 days. Based on the results, the highest grafting success was observed with omega (84.33%) followed by side stub (41.89%) and whip tongue (24.31%) grafting, respectively. Significant variations were also observed in graft take and scion growth. The differences among walnut cultivars (scion) on grafting take and scion growth were not significant. However, the scions x grafting methods interaction was significant and 'Hartley' variety grafted by omega method showed the highest graft take (88.44%) among all combinations. A significant positive correlation ($R^2 = 0.84$) was observed between the callus quality and graft takes in all grafting methods.

Key words: Callus formation, grafting techniques, graft survival, greenhouse, sawdust, walnut cultivars.

Introduction

Persian walnut (*Juglans regia* L.) is an important nut crop and still being propagated by seedlings in several countries including Iran, which resulted to high variability and poor crop quality (Vahdati, 2000). Selection and vegetative propagation of the superior walnut cultivars of different agroclimatic areas is the most efficient method for increasing the production and nut quality (Solar *et al.*, 2001). Vegetative propagation in walnut is more difficult in comparison with other fruit trees (Kuden and Kaska, 1997; Ozkan and Gumus, 2001) and low success has always been considered a drawback in massive propagation of superior walnut individuals (Ozkan and Gumus, 2001; Vahdati, 2003). Walnut grafting success were reported to be affected by several factors including graft technique, temperature, humidity, phenolic compounds, hormonal condition, nutrition of scion cultivars, and time of taking the scions (Mitrovic, 1995; Mehmet *et al.*, 1997).

Environmental conditions during and following grafting have major impact on callus formation in walnut (Millikan, 1971; Rongting and Pinghai, 1993; Avanzato and Atefi, 1997; Ebrahimi *et al.*, 2006). In Persian walnut, for successful grafting, temperature around the grafting point should be maintained at about 27 °C after grafting (Avanzato and Atefi, 1997; Germain *et al.*, 1997).

In walnut vegetative propagation, fluctuating temperatures and lack of sufficient humidity cause undesirable environment under field condition responisble for poor callus formation and grafting failure (Ebrahimi *et al.*, 2006), thus bench grafting methods are being usually preferred. The advantages of these techniques include:1) table grafting could be done under controlled conditions and gives a better result, 2) the operation could be done during winter and 3) grafting could be mechanized to increase labour productivity (Lantos, 1990; Tsurkan, 1990; Unal, 1995).

New methods using bench grafting techniques like hot cable or hot callus have been developed and used by different researchers (Avanzato and Atefi, 1997; Hartmann *et al.*, 2001), but requirement of more skillful workers and expensive facilities restrict their application in most nurseries. Therefore, the main goal of the present study was to evaluate the efficiency of modified bench grafting methods in order to decrease the high cost of production by grafting in walnut as well as to compare different walnut cultivars in terms of grafting success and scion growth.

Materials and methods

The experiment was conducted in Department of Horticulture, College of Abouraihan, University of Tehran during March 2006, to evaluate effect of factorial combination of three grafting methods (side stub, omega and whip tongue) and four walnut cultivars ('Z53', 'Hartley', 'Pedro' and 'Serr') on callus formation, grafting success and scion growth. The scions were stored at cool and moist condition, until they were used in grafting. The seedling rootstocks were taken in late January and selected for size and uniformity. The study was conducted using a factorial experiment on a complete randomized design with 12 treatments in three replications and 15 seedlings per plot.

All of the grafted combinations were carried out by the same person and standard methods were used for grafting as described by Hartmann *et al.* (2001) briefly as following. For side-stub grafting, the basal part of the scions were cut as wedge of about 2.5 cm and an oblique cut at angle of 20º to 30º was made into the basal part of the seedling's stem then scion were inserted, without any fastening material. In whip grafting, a sloping cut (2-3 cm in length) was made at the top of the rootstock, and then a second downward cut was made starting one-third of the distance from the tip to the base of the first cut. Similarly, a sloping cut

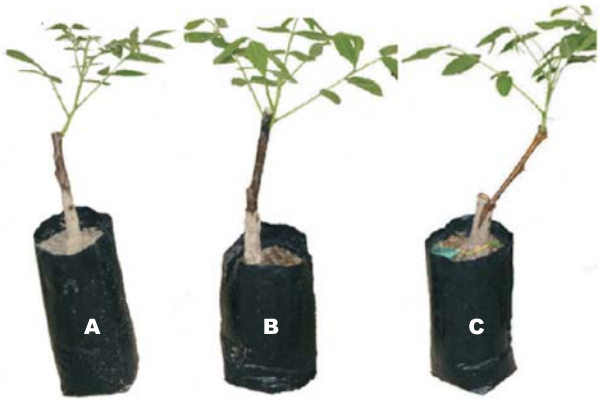

Fig. 1: A view of grafted plants after two months of grafting and transferring to pots. A) Whip tongue grafting method, B) Omega grafting, and C) Side stub grafting method.

was made at the base of the scions to the same length as the cut on the rootstock. Also a second cut was made under the first cut similar to the stock. Finally, the graft compounds were slipped together to be interlocked, and were tied by rubber tape tightly without waxing. The omega grafting method was performed by omega grafting machine (OMEGA-STAR Company of Germany) without any fastening similar to the side stub grafting method.

Grafted plants were maintained in a greenhouse at 26-28 ºC inside the sawdust with the moisture content of 85-90% for 21 days. After completing callusing period, data were recorded for amount of callus formation based on a visual scale of one to four in which 1= poor, 2= medium, 3= high and 4= very high callusing. Subsequently, the percentage of callus formation, graft take, and scions growth were measured in each treatment. The grafted plants then were transferred to the black polyethylene pots (20 × 30 cm) containing sand: soil: leaves compost mixture (1:2:1 w/w), and were put in a humid greenhouse for about three months (Fig. 1). They were brought to the shade house in September, 2007, before transplanting to the field condition.

The collected data were analyzed by SAS statistical software (SAS Institute, North Carolina, USA) and means compared using Duncan's multiple range test (DMRT) method. Statistical significance indicates means difference ($P \leq 0.05$).

Results and discussion

The results showed that different grafting methods had significantly different effects on callus quality, graft take and grafting survival (Table 1). Among the methods, omega grafting showed the highest callus quality (2.5 from 4), higher graft take (67.77%) and grafting survival (84.33 %) as well as higher scion growth (12.9 cm) followed by side stub and whip grafting. Percentage of graft takes in side stub and whip tongue methods were 58.88 and 19.44%, respectively. However, percentages of grafting survival were about 41.89% in side stub and 24.31% in whip tongue methods (Table 1).

As demonstrated in Fig. 2, percentage of graft take shows a highly positive correlation with callus quality at different grafting methods and cultivars. This is in agreement with the results of Rongting and Pinghai (1993) that callus quality and amount of callus formation plays an important role in the grafting success.

In this experiment, the percent of grafting success in the most

Table 1. Effects of grafting methods on callus quality, graft take, survival and scion growth

Grafting type	Callus quality[A]	Graft takes (%)	Graft survival (%)	Scion growth (cm)
Omega	2.53a[B]	67.77a	84.33a	12.9a
Side stub	2.28a	58.88a	41.89b	5.38b
Whip tongue	0.97b	19.44b	24.31b	3.06b

[A]Values are means of callus scoring rating from 1 (low callus) to 4 (very good callus). [B]Means with different letters in each column are significantly different at $P \leq 0.05$.

efficient treatment was similar to Lantos (1990) who obtained 80% of grafting success using bench grafting method. Among three methods of grafting, omega grafting showed the best result considering all of studied characters which is consistent with Solar *et al.* (2001).

Significant differences was observed among the cultivars in terms of callus quality and graft survival, but the effect of variety on grafting take or scion growth was not statistically significant (Table 2). This is in contrary with the result of Rongting and Pinghai (1993) and Mitrovic *et al*. (1997) who reported the

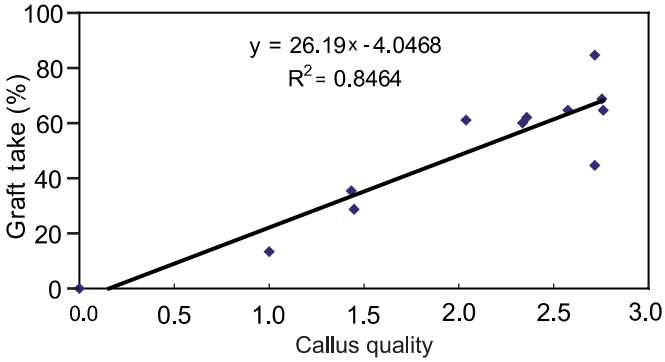

Fig. 2. Correlation between callus quality and graft take in different grafting methods.

significant effect of walnut cultivars on graft take. Although scion cultivar could affect grafting result, but it seems that it is mainly affected by scion quality which is a management related issue rather than their genetic makeup (Rezaee and Vahdati, 2008).

The interaction of grafting methods and variety on graft take, grafting survival and scion growth were significant (Table 3). The highest graft take (88.44%) was achieved by omega grafting of 'Hartley' and the lowest graft take by whip tongue grafting of 'Z53' and 'Hartley' cultivars with 0.00 and 13.33 %, respectively. The highest scion growth (14.66 cm) was in ' Z53' variety with omega grafting method and lowest scion growth (2.22 cm) was obtained from 'Serr' variety with side stub grafting method. The highest graft survival (96.67 and 90.91%) was obtained by grafting of 'Z53' and 'Pedro' cultivars using omega and side stub grafting methods, having the highest score of callus quality of 2.76 and 2.75, respectively

Table 2. Callus quality, graft take, survival and scion growth in different walnut cultivars

Cultivar	Callus quality[A]	Graft takes (%)	Graft survival (%)	Scion growth (cm)
'Serr'	2.24a[B]	45.92a	48.61ab	5.82a
'Pedro'	2.08a	54.07a	73.27a	8.16a
'Hartley'	2.01a	52.59a	42.22ab	8.57a
'Z53'	1.37b	42.22a	36.67b	5.90a

[A]Values are means of callus scoring ratings from 1 (low callus) to 4 (very good callus). [B]Means with different letters in each column are significantly different at $P \leq 0.05$.

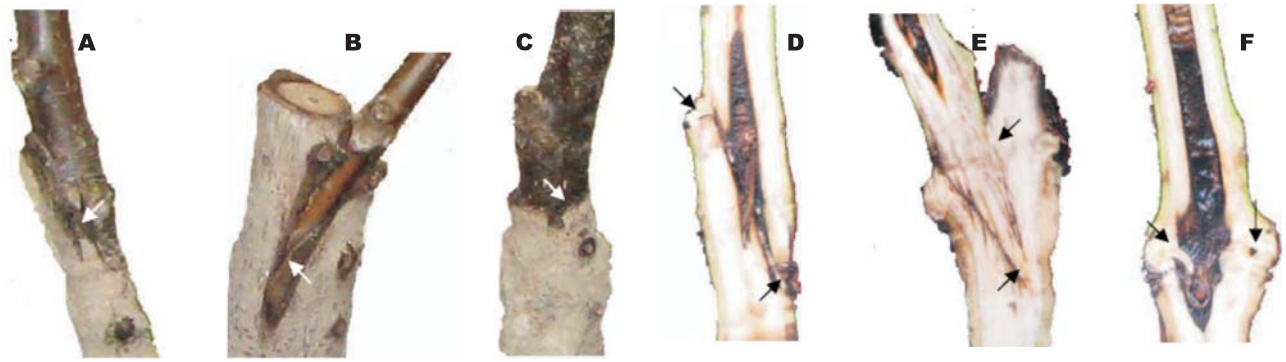

Fig. 3. Interlocking and quality of callus bridge formation between rootstock and scion in the studied grafting methods. A, D) Whip tongue method, B, E) Side stub method, C, F) Omega method

The results of this study were different than Ozcan and Gumus (2001) who found the highest success by using whip tongue grafting method. It could be probably related to the experimental condition; so that tight binding of the grafting place by rubber tape in our experiment, resulted to limitation in aeration and blockage of the phloem sap transportation under the graft cover. Adequate aeration and auxins have an important roles on callus formation and grafting success (Ronging and Pinghai, 1993; Vahdati, 2000; Hartmann *et al.*, 2001; Rezaee and Vahdati, 2008).

Interlocking and quality of callus formation between rootstock and scion in the omega and side stub grafting methods were approximately similar. Despite a good interlocking of grafting compound, as shown in Fig. 3, formation of callus bridge was restricted in whip tongue grafting method.

Considering the difficult-to-graft nature of walnuts and higher cost of specialized facilities used in methods like hot cable and hot callus (Avanzato and Atefi, 1997; Avanzato, 2001) the results of this experiment are comparably promising, and represents a good alternative method to propagate walnut cultivars under partially controlled condition. Further research is needed for improvement of grafting techniques and condition to obtain more uniform grafting success.

Table 3. Interaction of grafting methods × cultivar on callus quality, graft take, survival and scion growth

Grafting × variety	Callus quality[A]	Graft take (%)	Graft survival (%)	Scion growth (cm)
Omega × 'Z53'	2.76a[B]	64.44ab	96.67a	14.66a
Omega × 'Hartley'	2.71a	88.44a	80abc	14.34a
Omega × 'Pedro'	2.07ab	57.78bc	81.67abc	11.07ab
Omega × 'Serr'	2.57a	64.44ab	79.00abc	10.91abc
Side stub × 'Z53'	1.36cb	13.33d	11.11c	3.067bcd
Side stub × 'Hartley'	2.33a	60.00ab	30.00cd	7.44abcd
Side stub × 'Pedro'	2.75a	68.89ab	90.91ab	8.82abc
Side stub × 'Serr'	2.71a	44.44bcd	33.33bcd	2.22abc
Whip tongue × 'Z53'	0.00d	0.00f	0.00d	0.00d
Whiptongue ×'Hartley'	1.00c	13.33ef	16.67d	3.93bcd
Whip tongue × 'Pedro'	1.43bc	35.56cb	41.22abcd	3.98bcd
Whip tongue × 'Serr'	1.45bc	28.89ed	33.33bcd	4.33bcd

[A]Values are the means of callus scoring ratings from 1 (low callus) to 4 (very good callus). [B]Means with different letters in each column are significantly different at $P \leq 0.05$.

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