IJANS

International Journal of Agricultural and Natural Sciences
E-ISSN: 2651-3617 13(1): 1-9, 2020
Research Article

EFFECT OF DIFFERENT STORING TIMES ON GRAFT SUCCESS IN BENCH GRAFTED WALNUT PLANTS

Ercan Er[1,a], Ümit Serdar [2,b], Burak Akyüz[2,c,*], Erol Aydın[1,d]

[1]*Black Sea Agricultural Research Institute, Samsun, Turkey*
[2]*Ondokuz Mayıs University, Faculty of Agriculture, Department of Horticulture, Samsun, Turkey*

**Corresponding Author:*
E-mail: burak.akyuz@omu.edu.tr

(Received 29th March 2020; accepted 22th April 2020)

a: https://orcid.org/0000-0002-0188-8967, b: https://orcid.org/0000-0003-4703-6927, c: https://orcid.org/0000-0001-7356-776X, d: https://orcid.org/0000-0002-2710-4075

ABSTRACT. This study aimed to determine how long grafted walnut plants can be stored before transplanting them to the field. The experiment was conducted between 2012 and 2013 at Black Sea Agricultural Research Institute of Samsun, Turkey. One-year old seedlings of J. regia and scion woods of 'Chandler' cultivar were used as plant materials. The plants were grafted with whip-tongue, chip budding and Mr. Cherny grafting methods. In the study, two different storing treatments (heat & cold room treatment and cold room treatment) and four storing times (3, 6, 9 and 12 weeks) were tested. Graft success (%) was determined at the end of the vegetation period. The study was established with the experimental design of split - plots in random blocks with 3 replications. In each replication 18 plants were used. The highest graft success was obtained from whip-tongue grafted plants that subjected to the heat & cold room treatment and stored for 9 weeks by 83.3 % in 2013. Besides, it is determined that bench grafted walnut plants can be stored for 12 weeks after grafting. However, the rate of graft success decreases due to the increase in storing time.

Keywords: *Callus formation, J. regia, planting time, temperature*

INTRODUCTION

Walnut can be propagated by vegetative or generative methods. In the generative method, propagation material is seed. However, the biggest disadvantage of propagation by seed is the new plant can be different than the mother plant. Due to this disadvantage of generative propagation, vegetative propagation methods are commonly used for plant propagation. Among vegetative propagation methods, grafting is mostly preferred. Grafting of walnut requires more care than other fruit types [1, 2, 3, 4, 5, 6, 7]. Grafting success of walnut is affected by multiple factors such as cultivar, quality of the scion wood and rootstock, xylem exudation, collection time of the scion wood, grafting time and method, air temperature and relative humidity before and after grafting [1, 5, 8, 9, 10, 11]. Amongst them, the temperature is one of the major factors affect graft success of walnut. Many researchers reported that walnut does not form any callus below 20°C and the optimum temperature for callus formation is between 25 and 27°C [10, 12, 13, 14, 15]. This requirement for callus formation limits the grafting period for a short time. In ecologically unsuitable conditions, to extend the grafting period, grafting is performed in controlled indoor conditions [16].

There are two different indoor grafting applications. The first method is called as "Hot callusing at grafting point" method, which only heats graft union [2, 14, 16, 17, 18, 19],

the second method is called as “Classical method” that heats whole of the room to 25-28°C [20, 21, 22, 23]. In these two methods, most of the researchers transfer grafted plants into the greenhouse or open field immediately after graft healing. In the open field, unsuitable ecological conditions can cause graft failures. On the other hand, in the greenhouse, grafted plants should be potted and then should transferred to the open field. According to the pot size, potted plants generally have limited root area. So, it affects plant quality [24]. To overcome this problem grafted plants should be stored in proper condition and then should be transferred into the open field.

This study was planned to find out how long indoor grafted walnut plants can be stored before planting them into the open field.

MATERIALS AND METHODS

Material

This study was carried out between 2012 and 2013 at Black Sea Agricultural Research Institute in Samsun, Turkey. In the study, ‘Chandler’ cultivar was used as scion and one-year old Juglans regia seedlings as rootstocks. The scion woods were collected in January and they were stored at 4°C until grafting date.

Methods

Graftings were performed as bench grafting on 20-22 February in both years. Whip-tongue, chip budding, and Mr. Cherny grafting methods were used in the study. In Mr. Cherny grafting method a plastic material was placed between scion and rootstock (Fig. 1).

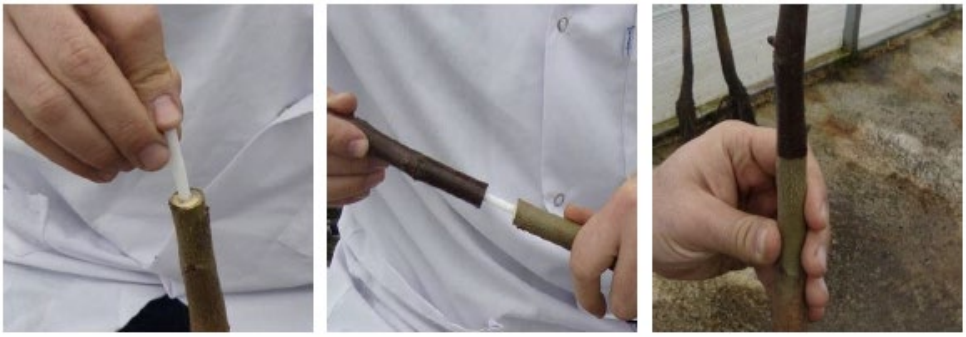

Fig. 1. *Application steps of Mr. Cherny grafting method*

Scion and rootstock were wrapped with plastic tape to hold them together. As an isolation material, water-based white paint was applied to the tip of the scion wood in whip-tongue and Mr. Cherny method and the tip of rootstocks in the chip budding method. In both years, graft union and scion woods were wrapped with parafilm.

Storing treatments

In 2012, grafted plants were put into wooden boxes as horizontally and they were completely covered with wet perlite. The top of the boxes was covered with polyethylene bags to avoid loss in moisture from the perlite (Fig. 2). On the other hand, in 2013 grafted plants were planted in plastic bags only their roots were covered with wet perlite (Fig. 3).

Fig. 2. *Storing of grafted walnut plants in a wooden box covered by polyethylene bag in 2012*

Fig. 3. *Storing of grafted walnut plants in perlite at the heat & cold and cold room in 2013*

Indoor grafted plants were subjected to two different storing treatments. In the first treatment, grafted plants were stored in a climatic room (24-27°C air temperature) for three weeks then they were transferred into a cold room (2-4°C temperature and 90 % humidity) and stored them until planting date (heat & cold room). In 2013, some of the grafted plants started to sprout from the scion wood. So, before transfer to the cold room whole of the plant was covered with slightly wet perlite within plastic bags. In the second treatment, grafted plants were stored in the cold room (2-4°C temperature and 90 % humidity) until the planting date (cold room). Air temperature and relative humidity of

the rooms were recorded by a data logger (KIMO 310). The temperature of the perlite medium was measured by a soil thermometer (measuring range -20°C to +50°C).

Storing times of grafted plants

After storing treatments, different storing times (about 3, 6, 9 and 12 weeks) were tested. For this aim, in 2012 and 2013 grafted plants were planted to the open field on 15 March, 5 April, 25 April and 15 May (3, 6, 9 and 12 weeks storing time, respectively). Grafted plants were planted in the open field by 20 x 150 cm spacing.

Study Design and Data Analyses

Graft success (%) was determined based on the number of grafts that survived at the end of the vegetation period. The study was established with the experimental design of split - plots in random blocks with 3 replications. In each replication 18 plants were used. 'JMP 17' statistic program was used to evaluate the data. $\sqrt{(x+1)}$ transformation was applied to the data due to 0% values [25]. The significance level of the differences between the means was determined by Duncan Multiple Range Test ($p \leq 0.05$).

RESULTS AND DISCUSSION

In 2012, graft success was ranged between 0 to 62.5 % according to the grafting method, storing treatment and storing time (Table 1). The best graft success was obtained from the plants propagated by chip budding method which were stored in the cold room for 3, 6 and 9 weeks (54.2 %, 62.5 %, and 58,7 % respectively) (Table 1).

Table 1. *Effect of storing treatments x grafting methods x storing time interaction on graft success (%) in 2012*

Storing Treatments	Grafting Methods	Storing Time				Mean
		3 weeks	6 weeks	9 weeks	12 weeks	
Heat & cold	Whip-tongue	9.4 de*	8.3 de	15.6 c	14.6 cd	**12.0 C****
	Chip budding	9.4 de	6.3 e	15.6 c	2.1 f	**8.3 C**
	Mr. Cherny	2.1 f	2.1 f	0.0 g	0.0 g	**1.0 D**
Cold	Whip-tongue	18.8 c	37.5 b	37.5 b	10.4 c-e	**26.0 B**
	Chip budding	54.2 a	62.5 a	58.7 a	2.1 f	**44.3 A**
	Mr. Cherny	0.0 g	0.0 g	0.0 g	0.0 g	**0.0 D**

* Difference between the means of storing treatments x grafting methods x storing time interaction shown in the same column with the same lower case is not statistically significant ($P \leq 0.05$).

** Difference between the means of storing treatments x grafting methods interaction shown in the same column with the same capital letter is not statistically significant ($P \leq 0.05$).

According to grafting methods chip budding (26.3 %) and whip-tongue (19.0 %) grafting methods gave the best results (Table 2). Storing times statistically affected graft success. The worst result was obtained from 12 weeks of storage.

Table 2. *Effect of grafting methods x storing time interaction on graft success (%) in 2012*

Grafting Methods	Storing Time				Mean
	3 weeks	6 weeks	9 weeks	12 weeks	
Whip-tongue	14.1 c*	22.9 b	26.6 ab	12.5 c	**19.0 A*** **
Chip budding	31.8 ab	34.4 ab	37.0 a	2.1 d	**26.3 A**
Mr. Cherny	1.0 de	1.0 de	0.0 e	0.0 e	**0.5 B**
Mean	**15.6 A****	**19.4 A**	**21.2 A**	**4.9 B**	

* Difference between the means of grafting methods x storing time interaction shown in the same column with the same lower case is not statistically significant (P≤0.05).
** The difference between the mean of the grafting methods indicated by the same capital letter in the same line is not statistically significant (P≤0.05).
*** The difference between the mean of the storing times indicated by the same capital letter in the same column is not statistically significant (P≤0.05).

Contrast to the literature the plants subjected to cold room treatment had better graft success than heat & cold room treatment (Table 3).

Table 3. *Effect of storing treatments x storing time interaction on graft success (%) in 2012*

Storing Treatments	Storing Time				Mean
	3 weeks	6 weeks	9 weeks	12 weeks	
Heat & cold	7.0 c*	5.6 cd	10.4 c	5.6 de	**7.2 B****
Cold	24.3 b	33.3 a	31.9 a	4.2 e	**38.7 A**

* Difference between the means of storing treatments x storing time interaction shown in the same column with the same lower case is not statistically significant (P≤0.05).
** The difference between the mean of the storing treatments indicated by the same capital letter in the same column is not statistically significant (P≤0.05).

As mentioned before, in 2012 after grafting, the whole of the plant was covered with perlite and stored in the wooden boxes until planting date. Perlite can hold 3–4 times its weight in water [26]. Perlite is a good material to keep scion woods or seeds alive in the cold room. However, in this case, high moisture level and temperature (about 24-27 °C) could have been damaged the graft area. Also, the graft area could be damaged mechanically when removing plants from the wooden boxes. Due to these negative results, in 2013 plants roots were only covered with wet perlite and placed vertically. Similar to the results, Akca et al. [27] obtained higher graft success (32.5 %) by using poplar sawdust as a cover material than perlite (20 %). Dehgan et al. [13] compared the effect of perlite and sawdust as a cover material on graft success. The sawdust had nearly two times better graft success compared to perlite.

In 2013, graft success was ranged between 0 to 83.3 % according to the grafting method, storing treatment and storing time (Table 4). The highest graft success was obtained from whip-tongue grafted plants that subjected to the heat & cold room treatment and stored for 9 weeks by 83.3 %.

Table 4. *Effect of storing treatments x grafting methods x storing time interaction on graft success (%) in 2013*

Storing Treatments	Grafting Methods	Storing Time				Mean
		3 weeks	6 weeks	9 weeks	12 weeks	
Heat & cold	Whip-tongue	77.1 ab*	68.8 a-c	83.3 a	62.5 a-c	**72.9 A****
	Chip budding	39.6 ef	54.2 cd	33.3 ef	29.2 fg	**39.1 B**
	Mr. Cherny	0.0 k	4.2 ı	0.0 k	2.1 ı	**1.6 C**
Cold	Whip-tongue	62.5 a-c	43.8 de	14.6 h	18.8 gh	**34.9 B**
	Chip budding	2.1 ı	0.0 k	0.0 k	0.0 k	**0.5 C**
	Mr. Cherny	0.0 k	0.0 k	0.0 k	0.0 k	**0.0 C**

* Difference between the means of storing treatments x grafting methods x storing time interaction shown in the same column with the same lower case is not statistically significant (P≤0.05).
** Difference between the means of storing treatments x grafting methods interaction shown in the same column with the same capital letter is not statistically significant (P≤0.05).

In this year, the highest graft success was obtained from the whip-tongue method by 53.9 % (Table 5). Graft success also changed according to the storing time. The highest graft success was obtained from 3 and 6 weeks stored plants by 30.2 % and 28.5 % respectively (Table 5).

Table 5. *Effect of grafting methods x storing time interaction on graft success (%) in 2013*

Grafting Methods	Storing Time				Mean
	3 weeks	6 weeks	9 weeks	12 weeks	
Whip-tongue	69.8 a*	56.3 b	49.0 bc	40.6 c	**53.9 A*****
Chip budding	20.8 e	27.1 de	16.7 ef	14.6 f	**19.8 B**
Mr. Cherny	0.0 h	2.1 g	0.0 h	1.0 gh	**0.8 C**
Mean	**30.2 A****	**28.5 A**	**21.9 B**	**18.8 B**	

* Difference between the means of grafting methods x storing time interaction shown in the same column with the same lower case is not statistically significant (P≤0.05).
** The difference between the mean of the grafting methods indicated by the same capital letter in the same line is not statistically significant (P≤0.05).
*** The difference between the mean of the storing times indicated by the same capital letter in the same column is not statistically significant (P≤0.05).

This year, better graft success results were obtained from heat & cold room treatment than cold room treatment. In heat & cold room treatment graft success was determined as 37.9 % and in the cold room as 11.8 % (Table 6). In cold room treatment except 3 weeks stored plants propagated by chip budding method, only graft success was obtained from the whip-tongue grafting method. However, graft success was decreased due to the increase in storing time (Table 4).

Table 6. *Effect of storing treatments x storing time interaction on graft success (%) in 2013*

Storing Treatments	Storing Time				Mean
	3 weeks	6 weeks	9 weeks	12 weeks	
Heat & cold	38.9 ab*	42.4 a	38.9 ab	31.3 b	**37.9 A****
Cold	21.5 c	14.6 d	4.9 e	6.3 e	**11.8 B**

* Difference between the means of storing treatments x storing time interaction shown in the same column with the same lower case is not statistically significant (P≤0.05).

** The difference between the mean of the storing treatments indicated by the same capital letter in the same column is not statistically significant (P≤0.05).

In 2013, because of the negative effect of perlite in the graft area, only roots were covered with perlite. This led the graft area to be subjected to air flow in the cold room. Reil et al. [28], Şen [29] and Hartmann et al. [10] suggested that walnut need high temperature (25-27 °C) for graft formation. Also, it has been reported that no callus formation was observed on the plants which were subjected 10 °C and 16 °C for three weeks [15]. In this period, it is important to keep scion woods alive before callus formation completed [11, 15]. This can also explain the low graft success on chip budding and Mr. Cherny grafting methods. In these methods, the graft formation area is smaller than the whip-tongue grafting method. So, they could be much more affected than cold compared to the whip-tongue grafting method. On the other hand, in heat & cold room treatment, before the transfer to the cold room, plants were covered with perlite. This could help to prevent air flow to damage the graft area.

Amongst grafting methods, Mr. Cherny grafting method gave the worst results in both years. Akyüz and Serdar [24] also indicated that they had low graft success with Mr. Cherny grafting method. In 2012 statistically, there was no difference between chip budding and whip-tongue grafting methods on graft success. In 2013 the best graft success was obtained from the whip-tongue grafting method. Also, similar results were obtained from different studies that compared chip budding and whip-tongue grafting methods [11, 21, 30].

In heat & cold room treatment the climatic room's temperature was set between 24-27 °C for three weeks (classic method). On the other hand, Achim and Botu [30] compared the classical method with hot callusing at the grafting point. They had better graft success with hot callusing at grafting point compared to the classic method (81.3 % and 59.6 % respectively). Also, in another study effect of hot callusing at graft point on graft success was tested. In the first-year, graft success was determined as 80.9 % and in the second year as 92.4 % [18].

CONCLUSION

As a conclusion, the highest graft success was obtained from whip-tongue grafted plants which subjected to the heat & cold room treatment and stored for 9 weeks by 83.3 % in 2013. Besides, grafted walnut plants can be stored for 12 weeks after grafting. However, the rate of graft success decreases due to the increase in storing time. Graft success can be increased by heating treatment. If there is no possibility for heating, grafts could be stored directly in the room conditions or cold room. In this case, graft formation starts after planting to open field. In both cases, the viability of graft unions or scion shoots should be maintained. For this aim, covering the whole of the grafted plant with

perlite is not a desirable method due to high temperature and moisture in a heated room or mechanical damage when removing plants from boxes. More studies should be done with hot callusing at graft point. Whip-tongue grafting method had better graft success compared to other methods. However, this method requires more care than other methods. So further studies should be done with other grafting methods.

Acknowledgement. The authors are grateful to the Ondokuz Mayıs University BAP Commission (Project number PYO.ZRT.1904.12.006) for funding.

REFERENCES

[1] Kuniyuki, A. H., Forde, H. I. (1985): Propagation. In: Ramos, D.E. (ed.) Walnut Orchard Management, Division of Agriculture and Natural Resources, California, USA.
[2] Avanzato, D., Tamponi, G. (1988): The Effect of Heating of Walnut Graft Unions on Grafting Success. Acta Horticulturae 227: 79- 83.
[3] Coggeshall, M. V., Beineke, W. F. (1997): Black Walnut Vegetative Propagation: The Challenge Continues. - In: Sambeek Van, J.W. (ed.) Knowledge for the future of black walnut, U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station. USA.
[4] Akca, Y. (2005): Walnut Cultivation. Tarım ve Köyişleri Bakanlığı Yayın Daire Başkanlığı Matbaası, Ankara. (In Turkish).
[5] Gandev, S. (2007): Budding and Grafting of the Walnut (*Juglans regia* L.) and their Effectiveness in Bulgaria. - Bulgarian Journal of Agricultural Science 13: 683-689.
[6] Ahmed, N., Singh, S. R., Srivastava, K. K., Shagoo, P. A., Hayat, S. (2012): Effect of Different Environments, Grafting Methods and Times on Sprouting, Graft Success and Plant Growth of Walnut (*Juglans regia*). Indian Journal of Agricultural Sciences 82(12): 1022-1026.
[7] Majd, S. R., Kourosh, V., Mahmoud, R. R., Mostafa, A. (2019): Exploring Combinations of Graft Cover and Grafting Method in Commercial Walnut Cultivars. International Journal of Fruit Science 19:4, 359-371.
[8] Rongting, X., Pinghai, D. (1993): A study on the uniting process of walnut grafting and the factors affecting. Acta Horticulturae 311: 160-170.
[9] Rezaee, R., Vahdati, K. (2008): Introducing of a simple and effective procedure for topworking Persian walnut tree. Journal of American Pomological Society 62: 21-26.
[10] Hartmann, H. T., Kester, D. E., Davies, F. T., Geneve, R. (2014): Plant Propagation: Principles and Practices, Pearson Prentice Hall, New Jersey, USA.
[11] Akyüz, B., Serdar, U., (2017): The Relationship Between Environmental Factors and Rootstock Growth Stage with Graft Success in Walnut. Journal of the American Pomological Society 71(4): 220-225.
[12] Erdogan, V. (2006): Use of Hot Callusing Cable in Walnut Propagation. Acta Horticulturae 705: 313-317.
[13] Dehgan, B., Vahdati, K., Rezaee, R., Hassani, D. (2010): Walnut Grafting Success as Affected by Different Grafting Methods, Cultivars and Forcing Treatments. Acta Horticulturae 861: 345-352.
[14] Soleimani, A., Rabiei, V., Hassani, D. (2010): Effect of Different Techniques on Walnut (*J. regia* L.) Grafting. - Journal of Food Agriculture & Environment 8(2): 544-546.
[15] Öztürk, G. (2018): Farklı Sıcaklıkların Cevizde Aşı Başarısına Etkisi. Ondokuz Mayıs Üniversitesi, Fen Bilimleri Enstitüsü. Samsun, Turkey.

[16] Gandev, S. (2009): Propagation of Walnut (*Juglans Regia* L.) under Controlled Temperature by the Methods of Omega Bench Grafting, Hot Callus and Epicotyl Grafting. Bulgarian Journal of Agricultural Science 15(2): 105-108.
[17] Atefi, J. (1997): Comparison of Hypocotyle and Hot Callus Cable Graft with Traditional Grafting Method. Acta Horticulturae 442: 309–313.
[18] Erdogan, V. (2005): Studies on the Use of Hot-Callusing Technique in Walnut Propagation. Bahçe 34(1): 225-230 (In Turkish).
[19] Avanzato, D. (2009): Training on Hot Callusing Technique Applied to Walnut grafting. Walnut propagation training short course, COST Action 873, Spain.
[20] Kazankaya, A., Şen, S. M., Tekintaş, F.E. (1998): Grafting Studies on Walnut. East Anatolia Agricultural Congress 1: 608-615. (In Turkish).
[21] Ozkan, Y., Gumus, A. (2001): Effects of Different Applications on Grafting Under Controlled Conditions of Walnut (*Juglans regia* L.). Acta Horticulturae 544: 515-520.
[22] Bayazit, S., Burhanettin, İ., Küden, A. (2005): Determination of Grafting Times and Methods of Walnut Under Adana Ecological Conditions. Bahçe 34(1): 231-234. (In Turkish).
[23] Paunovic, S. M., Miletic, R., Mitrovic, M. (2010): Walnut Grafting Success as Affected by Strafication. Acta Agriculturae Serbica 30: 165-172.
[24] Akyüz, B., Serdar, U. (2017): Tüplü Ceviz Fidanı Üretiminde Farklı Sürgün Aşı Yöntem ve Zamanlarının Aşı Başarısına Etkisi. Bahçe 46 (2): 267–272 (In Turkish).
[25] Tosun, F. (1991): Tarımda Uygulamalı İstatistik Metotları. Ondokuz Mayıs Üniversitesi Ziraat Fakültesi Ders Notları 1:256 (In Turkish).
[26] Papadopoulos, A., Bar-Tal, A., Silber, A., Saha, U., Raviv, M. (2008): Inorganic and Synthetic Organic Components of Soilless Culture and Potting Mixes. 10.1016/B978-044452975-6.50014-9.
[27] Akca, Y., Yılmaz, S., Bolsu, A., Uysal, F. (2005): Investigation on Applicability of Bare Root Grafting Technique as New Indoor Grafting Model In Walnut Plant Production. Bahçe 34(1): 235- 239 (In Turkish).
[28] Reil, W. O., Leslie, C. A., Forde, H. I., McKenna, J. R. (1998): Propagation. In: Ramos, D.E. (ed.) Walnut Orchard Management, Division of Agriculture and Natural Resources, California, USA.
[29] Şen, S. M. (2011): Ceviz Yetiştiriciliği, Besin Değeri ve Folklorü. 4. Baskı ÜÇM Yayınları, Ankara (In Turkish).
[30] Achim, G., Botu, I. (2001): Results in Walnut Propagation by Using Different Methods. Acta Horticulturae 544: 503-509.