



The Effect of Whip Grafting Method on Grafting Success and Plant Development in Some Cultivars of Anatolian Walnut (*Juglans Regia* L.)

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Abstract

The objective of this study was to determine the effects of whip grafting methods on graft success and plant development (the length and diameter of graft shoot) in open rooted walnut plant production. In the study, scion woods of “Chandler, Maraş-18, Şebin, Kaman-1, Kaman-2, Bilecik and Şen-2” walnut cultivars and one year old walnut rootstocks were used. The study was carried out in the open field and under shaded greenhouse conditions. Walnut seedlings were dug out in February and were taken burial in soil. Seedlings were grafted with whip grafting method in a room and planted in March. After planting, graft union was covered by soil within plastic bag and grafting wax was applied to upper side of scion wood. However, some graftings with Chandler clones were planted in the plastic greenhouse. Thus, it was also aimed to determine the effects of greenhouse condition on grafting success and plant development in walnut. Grafting success was changed between 59.6% and 98.8%. The highest graft success was obtained from Chandler cultivar produced both in the greenhouse and open field. There were no statistical differences between open field and greenhouse conditions in this cultivar. However, better scion shoot length and diameter were obtained in the greenhouse.

Keywords: Whip grafting, open rooted plant, walnut, height, diameter.

Anadolu Cevizinde (*Juglans regia* L.) Dilcikli Aşı Yönteminin Farklı Ceviz Çeşitlerinde Aşı Başarısı ve Büyüme Üzerine Etkisi

Öz

Bu çalışmada çıplak köklü aşılı ceviz fidanı üretiminde dilcikli aşının farklı ceviz çeşitlerindeki aşı başarısı ve fidan gelişimi üzerine etkileri incelenmiştir. Araştırmada “Chandler, Maraş-18, Şebin, Kaman-1, Kaman-2, Bilecik ve Şen-2” ceviz çeşitlerine ait aşı kalemleri ve bir yaşlı ceviz anaçları (çöğür) kullanılmıştır. Anaç olarak kullanılacak 1+0 yaşlı ceviz (*Juglans regia* L.) anaçları, Şubat ayı içerisinde sökülüp ve gömüye alınmıştır. Fidanlar Mart ayı içerisinde dilcikli aşı yöntemi ile kapalı bir ortamda aşılanmış, aşılanan fidanlarda kalem ve anaç plastik kelepçe ile sabitlenmiş, aşılı fidanlar araziye dikilmiş ve aşı bölgesi polietilen poşet içerisine geçirilerek toprak ile kapatılmıştır. Kalem en üst kısmına aşı macunu sürülmüştür. Çalışma arazide çıplak köklü fidanlarda yürütülmüş, bununla birlikte Chandler çeşidi ile yapılan aşıların bir kısmı naylon sera içerisine dikilmiş, böylece sera ortamının aşı başarısı ve fidan gelişimi üzerine etkisi de belirlenmeye çalışılmıştır. Araştırmada çeşitlere göre aşı başarısı %59.6–98.8 arasında değişmiştir. En yüksek aşı başarısı açık alanda ve serada yapılan Chandler çeşidinde elde edilmiştir. Chandler çeşidi ile açık alanda ve serada yapılan aşılar aşı başarısı bakımından istatistiksel farklılık bulunamamıştır. Bununla birlikte aşı sürgünü boyu ve çapı bakımından en iyi fidan gelişimi serada yapılan aşılar elde edilmiştir.

Anahtar Kelimeler: Dilcikli aşı, çıplak köklü fidan, ceviz, fidan boyu, fidan çapı

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1. Giriş

Walnut, which has a fruit covered by a hard shell, is one of the most important plant species of our country. Our country, which is one of the gene centers of the walnut, is among the countries growing walnut (*Juglans regia* L.) for a long time (Şen 2011). Anatolian walnut (*Juglans regia* L.) that has an originally wide range of distribution has taken out of this area through various migrations and trade trains, and become a fruit species that is widely grown in many regions of the world except the tropical ones (Şen 2011). Being an important species in terms of agricultural forestry and being cultivated, the global production amount of walnut in 2012 was 3.418.559 tons of the world (FAO 2015) (Table 1) of which Turkey had a production of 194.298 tons on the 4th rank.

Table 1. Global Walnut Production (tons) (FAO, 2015).

Country	2001	2006	2009	2010	2012
China	252.347	475.455	979.366	1.284.350	1.700.000
Iran	168.031	265.000	463.000	475.000	450.000
USA	276.690	317.515	396.440	457.221	425.820
Turkey	116.000	129.614	177.298	178.142	194.298
Ukraine	55.130	68.750	83.890	87.400	96.900
Mexico	65.000	68.359	115.350	76.627	110.605
France	27.815	40.333	20.417	30.855	36.425
India	29.000	36.000	36.000	38.000	40.000
Romania	33.942	38.471	38.329	34.359	30.546
Total	1.336.123	1.771.441	2.646.663	2.989.107	3.418.559

In order to select the superior species in terms of yield and fruit quality, many studies have been carried out in Turkey (Yaviç 2000; Güven & Güleriyüz 2001; Serdar *et al.* 2001; Ünver & Çelik 2005; Beyhan 2005; Yıldırım *et al.* 2005; Akça & Köroğlu 2005; Yarılgaç *et al.* 2005; Oğuz & Aşkın 2007; Beyhan 2009; Şimşek & Osmanoğlu 2010; Çelik *et al.* 2011). As a result of those studies, 26 species have been registered (TTSM 2015). Besides the national species, also the foreign species have been taken under examinations in Turkey and 7 of them have been registered (TTSM 2015). In proportion to other species, graft development requires much more effort in walnut. The most important reasons of that are the high sensitivity to bleeding due to excessive juice pressure and the requirement of higher temperature for graft integration (Şen *et al.* 2011).

Propagation of walnut with grafting method is closely related with the climate conditions of the region. The climate conditions, where the grafted plants will be transferred after grafting, also affect directly the rate of living sapling. The aim of this study is to determine the success of whip grafting method in production of open-root walnut sapling in Samsun Directorate of Forestry's Bafra Forest Nursery and to determine the effects on sapling development. Moreover, it is also aimed to reveal the effects of whip grafting method on grafting success and development under open-air and greenhouse conditions.

2. Material ve Method

2.1. Material

This study was carried out in the application area and greenhouses (4x47m) of Bafra Forest Nursery in 2015 (Fig.1). Bafra district is located on the wide delta constituted by rich alluvial soil brought by Kızılırmak River. Bafra Plain is approximately 40 km in length and 20 km in depth, and also the largest lowland plain of Black Sea region. The center of district is 20 km away from Black Sea. Its altitude is 15- 20 m, and its coordinates are 35° 53' 43" E and 41° 33' 56" N. The district is covered by Black Sea in east and north, Alaçam district in west, and Kavak district in south. It is 50 km away from Samsun city. Its area is 175.000 ha. Bafra Plain covering Kızılırmak delta is covered by the mountains in south. Those mountains are the extensions of Canik Mountains. The highest point is Mount Nebyan with altitude of 1.224 m. Kızılırmak, the longest river of Turkey, reaches the plain by passing through those mountains. Bafra plain is totally constructed by Kızılırmak. Bafra Pamuklu Forest Nursery is 67 km away from Samsun, 17 km from Bafra district, 1km from Koşu village. Bafra Pamuklu Nursery has been established in 1962 on 354.50 da area for crossbreed poplar production in Kızılırmak delta, and started to distribute saplings in year 1964. The area of sapling plantation is 252.115 da. Currently, the range of sapling species widened. For instance, the sapling species produced in this plantation are generally black locust, maple, ash tree, plane, laurel, crossbreed poplar 77/51, stone pine, and etc. besides the walnut and mulberry trees as grafted species.



Figure 1. A view from walnut sapling production in the greenhouse.

2.1.1. Meteorological Data and Soil Properties

Annual relative humidity of Bafra district is higher than 70%. Especially in April and May, this ratio exceeds beyond 77-79%, and falls to 70% in December. The highest precipitation level is observed in November, while lowest one is observed in May. Annual level of precipitation is approximately 750-1000mm. The snowfall is seen rare and it doesn't continue for a long duration. No snow accumulates in coastal region. In inner regions, the snow depth increases. The coldest months in region are January (-5.3°C) and February (-5.8°C), while the hottest one is August (28.6°C). In this study, the soil samples taken from land and greenhouse were analyzed in Black Sea Agricultural Research Institute, and some of the properties of soil are presented in Table 2. The soil, where the saplings were grown, is clayey-sandy in nature, has neutral structure, and is classified in chalky and salt-free class. Potassium content of such soils having low level of phosphor and very low level of organic matter is high.

2.1.2. Rootstock and Grafting Material

Systematically, walnut is the member of *Juglandaceae* family and *Juglans* genera. Walnut species has 22 varieties spreading throughout the mild and subtropical climate regions of the world. Among those varieties, the most economically important one is *Juglans regia* L. In this study, the open-root 1+0 year-old walnut (*Juglans regia* L.) rootstocks produced by Bafra Forest Plantation of Samsun Forest Directorate were used, while “Chandler” walnut graft from İzmir and “Şebin, Maraş-18, Kaman-1, Kaman-2, Bilecik and Şen-2” walnut (*Juglans regia* L.) scions from Amasya were used. In selection of rootstock, the saplings having 2-2.5cm thickness and good condition in terms of development and root structure were preferred. Some of the characteristics of varieties used as graft are presented below.

Table 2. Soil characteristics of experiment parcels.

Characteristics	Land	Greenhouse
pH	7.12	6.90
Sand (%)	12.70	14.78
Clay (%)	35.32	34.42
Silt (%)	51.98	50.80
Lime (CaCO ₃ , %)	11.3	11.7
Total Salt (%)	0.015	0.028
Phosphor (P ₂ O ₅ , kg/da)	3.45	2.49
Potassium (K ₂ O, Kg/da)	59	70
Organic Matter (%)	0.97	0.81

2.2. Method

2.2.1. Growth of Rootstocks and Preparation for Scions

In this study, the open-root 1+0 year-old walnut (*Juglans regia* L.) rootstocks produced by Bafra Forest Plantation of Samsun Forest Directorate were removed in February by cutting the roots at 30 cm depth, and then taken into heel-in. Before grafting, the root cleaning was performed for rootstocks to form the taproot and hairy roots. In order to ensure thickness matching between rootstock and scion, rootstocks were cut at 15-20 cm height above root collar.

2.2.2. Procurement and Storage of Scions

In this study, 1-1.3 m Chandler shoots within moist sand and covered by nylon, which will be used as graft, were procured from İzmir on 26th February 2015, while 0.9-1.10 m shoots of other varieties were procured from Amasya on 22nd March 2015 in the form of covered with sack in a closed medium. The scions were taken from 1 year-old healthy shoots from outer parts of the trees. The grafts were kept within moist polyethylene in cold-storage at 4 ± 1 °C temperature and 95% moisture until the grafting time. The rootstocks were grafted with the whip grafting method between 17th and 29th March of 2015 in a closed space. The grafting operation was executed at 15-20 cm above the root collar. In the whip grafting method, the grafts containing 2-3 sprouts were used. Rootstocks and scions having same thickness were considered. 3-4 cm and inclined cut was made in rootstock and scion (Figure 2).



Figure 2. Placing the scion to rootstock in whip grafting method.

Grafted saplings were planted in open field on the following day after root cleaning, the grafting points of the saplings were covered with soil taken into tube, and the top point of grafts were closed with grafting wax. This study was continued with naked-root saplings in the field, while some of the saplings grafted with Chandler clone were planted within greenhouse in order to determine the effects of greenhouse medium on grafting success and sapling development (Figure 3).



Figure 3. Grafted walnut saplings and their plantation in the field.

The irrigation was performed via sprinkling method. 1 week after the plantation, the NPK compound fertilized was implemented in dose of 8-9 g per sapling. After grafted saplings started to come up, the shoots below the point of grafting were cut and the weeds were cleaned on a regular basis. When the length of grafted shoot reached at 15-20 cm (16th May 2015), the soil in polyethylene bag outside the grafting point was carefully removed, and the grafting point was bandaged with plastic grafting rope. Plastic grafting ropes were removed 1-1.5 months later by considering the developmental status of saplings. In 1st week of July, Biotrissol N 46% Organic Matter (Liquid Fulvic Acid) liquid fertilizer was implemented at the dose of 0.004- 0.005 L/sapling. By removing some of the leaves and fruits at the bottom parts of saplings in June-September period, better development of saplings was ensured (Figure 4).



Figure 4. Irrigation of walnut saplings and weed control.

2.2.3. Determining the parameters about grafting success

In order to determine the grafting success, following parameters were measured:

2.2.3.1. Scion Shoot Ratio (%)

After 2 months from grafting, the portion of the number of saplings having shoots from graft to the number of seedling grafted at the beginning was calculated.

2.2.3.2. Length of Scion Shoot (cm)

After the end of first development phase, the length from the level of graft, where the shoot stooled, to the tip of shoot was measured (Figure 5).

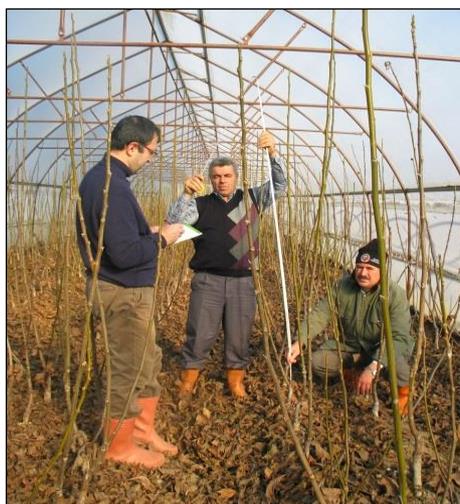


Figure 5. Length measurement of scion shoot of saplings.

2.2.3.3. Diameter of Scion Shoot (mm)

It is the diameter measured at 5 cm above the level of graft shoot at the end of the first development phase using a digital compass at 0.1 mm sensitivity.

2.2.3.4. Statistical analyses

The study was designed to be triplicated Randomized Blocks Research Pattern, and 30 graftings were implemented in each of repetitions. In different mediums experiment, the mean values were compared by Variance Analysis (ANOVA). As a result of statistical analyses, in order to detect the significant differences between the mean values and to mark the different ones with different letters in accordance with the level of difference, Duncan Multiple Range Test in same package software was used. In analyzing the data, SPSS package software was utilized.

3. Results and Discussion

3.1. Comparison of Different Clones in Sapling Propagation in Greenhouse and Open-Field

In this study, high significant difference ($P < 0.01$) was found between varieties in terms of grafting success and grafting shoot in sapling propagation production in greenhouse and open-field, while significant difference ($P < 0.05$) was found in terms of graft shoot diameter (Table 3).

Table 3. Grafting success (%), graft shoot length (cm) and diameter (mm) by the variety of walnut production in greenhouse.

Clones – Growth Mediums	Grafting success (%)	Grafting shoot length (cm)	Grafting shoot diameter (mm)
Chandler- Greenhouse	98.8 a*	155.9a	17.23a
Bilecik – Open field	59.6 c	88.3c	16.36ab
Chandler - Open field	93.2 a	117.4b	15.80ab
Kaman-1 - Open field	85.2 ab	49.1d	14.39ab
Kaman-2 - Open field	85.4 ab	43.6d	14.09b
Maraş-18- Open field	66.8 c	56.6d	13.80b
Şebin- Open field	72.6 bc	84.6c	16.19ab
Şen-2- Open field	74.3 bc	87.3c	14.77ab
Significance	$P < 0.01$	$P < 0.01$	$P < 0.05$

*No statistically significant difference between the mean values marked with same letter ($P < 0.01$).

The graft success (shoot ratio) of the study varied between 59.6% and 98.8%. The highest graft success was obtained in production of Chandler clone in greenhouse (98.8%), followed by Chandler, Kaman-2 and Kaman-1

varieties that were grown in open field. The lowest graft success was obtained in Bilecik (59.6%) clone. During the experiments, the graft shoot lengths of the varieties varied between 43.6 and 155.9 cm. The longest graft shoot was found in Chandler clone grown in greenhouse, followed by Chandler, Bilecik, Şen-2 and Şebin clones grown in open-field (155.9, 117.4, 88.3, 87.3 and 84.6 cm respectively). The graft shoot lengths of Kaman-1, Kaman-2 and Maraş-18 clones were much shorter. In grafting shoot diameters, where statistically significant differences were found, the thickest saplings were obtained from Chandler clone grown in greenhouse, followed by Bilecik, Şebin, Chandler, Şen-2, and Kaman-1 clones grown in open-field. The grafting shoot diameters of Kaman-2 and Maraş-18 clones were much lower.

3.2. Comparison of Different Varieties in Sapling Propagation in Field

In the study, high significant differences were found between the sapling production in the field in terms of grafting success and graft shoot length ($P<0.01$), while no significant difference was found in terms of graft shoot diameter (Table 4). Grafting success (shoot ratio) varied between 59.6% and 93.2%. The highest grafting success was obtained in Chandler variety with a value of 93.2%. This clone was followed by Kaman-2 and Kaman-1. The lowest grafting success was found in Bilecik (59.6%) clone. Throughout the study, the grafting shoot lengths varied between 43.6 and 117.4 cm. The longest graft shoot length was obtained from Chandler clone, followed by Bilecik, Şen-2 and Şebin clones (117.4, 88.3, 87.3 and 84.6 cm respectively). The graft shoot lengths of Kaman-1, Kaman-2 and Maraş-18 were much shorter. Although there was no statistically significant difference, the thickest graft shoot was obtained from Bilecik variety and the thinnest one from Maraş-18 clone.

Table 4. Graft success (%), graft shoot length (cm) and diameter (mm) by the clones of walnut production in open-field.

Clones	Grafting success (%)	Grafting shoot length (cm)	Grafting shoot diameter (mm)
Bilecik	59.6c*	88.3b	16.36
Chandler	93.2a	117.4a	15.80
Kaman-1	85.2ab	49.1c	14.39
Kaman-2	85.4ab	43.6c	14.09
Maraş-18	66.8c	56.6c	13.80
Şebin	72.6bc	84.6b	16.19
Şen-2	74.3bc	87.3b	14.77
Significance	$P<0.01$	$P<0.01$	Non-significant

*No statistically significant difference between the means marked with same letter ($P<0.01$).

3.3. Comparison of Sapling Production in Different Mediums

The grafts made with Chandler variety in different mediums; the grafting success, graft shoot length and graft shoot diameter in greenhouse medium were found to be higher than those in open field (Table 5). In this study, the graft success (shoot ratio) varied between 93.2% and 98.8%. The graft shoot lengths were found to be 155.9cm in greenhouse medium, 117.4cm in open-field, while graft shoot diameters were found to be 17.23mm in greenhouse and 15.80mm in open-field.

Table 5. Grafting success (%), graft shoot length (cm) and diameter (mm) of Chandler variety in different growth mediums.

Growth Medium	Grafting success (%)	Grafting shoot length (cm)	Grafting shoot diameter (mm)
Open-Field	93.2b*	117.4b	15.80b
Greenhouse	98.8a	155.9a	17.23a
Significance	$P<0.01$	$P<0.01$	$P<0.01$

* No statistically significant difference between the means marked with same letter ($P<0.01$)

In the study it was found that the grafting success was between 59.6% and 93.2% in grafts made in open-field. The main reason for these differences might be the result of scions (except Chandler), which were procured late, were not dormant. In Chandler variety, graft of which has been procured much earlier, a very high success rate (93.2%) was obtained. Hence, in a study of Akyüz (2014) in Samsun on production of tubed walnut sapling in open-field, the highest grafting success rates (91.7-100%) were obtained from whip grafts made before the

rootstocks grow up (March-April). Besides that, Barut (2001) achieved 48.6% success rate in rootstocks grafted in open-field in a whip-graft study in Bursa. Asghar et al. (2006) found the highest grafting success rate among whip and cleft grafting methods to be in whip graft (64.4%) made in 19th February in Pakistan. Achim et al. (2001), who compared grafted and whip grafting method in Romania, found the highest grafting success rate as 85% in whip graft. Özkan & Gümüş (2001), in their study on cleft, whip, and grafted grafts made between January and March in Tokat, reported that the highest success rate was obtained from whip graft, and the ratio of graft shoot was 66% in Tokat genotype and 70% in Yalova genotype. As stated by Akyüz (2014), the ecology of Samsun has a climate which is very suitable for walnut sapling production with the whip grafting. Although the length of graft shoot was longer in Chandler variety, those of other clones varied. For those clones, the scions could be procured just a few days before the implementation, and mild come-up was observed in some of clones. Those clones were affected from frost on 30th and 31st March 2015 considerably; graft tips of saplings dried and then developed another shoot again and constituted the graft by healing. In our study, the success of grafting in sapling production with Chandler variety in greenhouse was higher than in open-field. But, on the other hand, even if there was a statistically significant difference in statistical analysis comparing 2 implementations (ANOVA), no significant difference was found between production in greenhouse and in open-field in terms of grafting success. Considering only the graft success in whip grafting in open-field, it was determined that the use of greenhouse is not required under conditions of Samsun (Bafra). Akyüz (2014) has also reported that there was no statistically significant difference between grafted and tubes saplings grown in greenhouse and in open-field. From the aspect of length and diameter of graft shoot, the highest values were obtained in greenhouse medium (Tables 3 and 5). The reason of that may be the higher temperature in greenhouses, more photosynthesis of saplings, and more carbohydrate accumulation. Ebrahimi et al. (2006), in their study in grafting in open-field and greenhouse in June 2005 in Iran, have reported that the graft shoot in saplings grown in greenhouse was longer than those grown in open-field.

4. Conclusion

The study investigates the success of whip grafting method on the propagation of grafted and naked-root walnut saplings and its effect on sapling development. According to the results of this research, following suggestions can be given:

- It was determined that the climate of Samsun (Bafra) is suitable for open-field walnut sapling production in terms of grafting success and sapling development. For this reason, the advantage of the region for walnut propagation must be well evaluated.
- It has been shown that, in order to achieve successful sapling production, the grafts should be taken in dormant season.
- It has also been found that it has positive effects on sapling development and diameter and consequently naked-root grafted walnut sapling production can be executed in Samsun (Bafra) region without greenhouse.
- A successful walnut sapling production could be done by fixing the grafting point with plastic clamp band after grafting the naked-root walnut sapling, then covering with soil, and finally binding with plastic grafting rope 1-2 months after the shooting at grafting point. But those operations lead to high costs. In order to decrease this cost, the effects of tying the grafting point with plastic rope and then covering the graft with paraffin on grafting success and sapling development should be investigated.
- Whip grafting in plantation without uprooting the rootstocks should also be examined.
- Even if the success could be achieved in sapling production with whip grafting method, whip grafting is a very hard-to-implement method and it requires grafts with 2-3 sprouts. As an alternative to that, the studies on applicability of patch grafting or chip budding, which are easier and more affordable, in Samsun conditions in August-September period should be carried out.

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