ACTA SCIENTIARUM POLONORUM

Acta Sci. Pol. Hortorum Cultus, 16(5) 2017, 77–87

www.acta.media.pl ISSN 1644-0692 DOI: 10.24326/asphc.2017.5.8

ORIGINAL PAPER

Accepted: 10.05.2017

THE INFLUENCE OF VARIOUS METHODS AND BUDDING DATES ON PRODUCTION OF BLACK MULBERRY (*Morus nigra* L.) SAPLING

Hamdi Zenginbal

Abant Izzet Baysal University, Vocational Community College of Bolu, 14100 Bolu, Turkey

ABSTRACT

This research was conducted to determine the effects of five different budding dates and six budding methods on graft success and plant growth in black mulberry sapling production. The experiment was carried out in Bolu, Turkey, between 2014 and 2015. The black mulberry (*Morus nigra* L.) was grafted on two-year old white mulberry (*Morus alba* L.) seedling rootstocks in the nursery conditions. The highest bud take (88.33% and 85.0%, respectively to years) and sprouting rate (85.0% and 71.67%, respectively to years) were obtained with chip budding by hand performed on May 1 in both years. The highest mean shoot length (41.33 cm and 39.68 cm, respectively to years) and diameter (6.26 mm in both years) were obtained with inverted T-budding performed on April 15, 2014 and 2015. Among all parameters the best type of buddings was chip budding by hand, followed by T and inverted T-budding. The lowest results in all parameters were obtained with patch budding and chip budding by tool. Budding date on May 1 was the most appropriate and the results obtained were the highest in all parameters.

Key words: field nursery, graft success, propagation, maiden trees production

INTRODUCTION

Mulberry belongs to the genus *Morus* and family Moraceae [Özrenk et al. 2010]. Black mulberry (*Morus nigra*) is one of the 3 common mulberry species along with white mulberry (*Morus alba*) and purple mulberry (*Morus rubra*) [Elmacı and Altuğ 2002, Koyuncu 2004]. Black mulberries are used as fruit farming or ornamental plants, which makes the fabulous shade plants in home gardens or parks.

The mulberry can be propagated by sexual and asexual methods [Lu 2002, Anis et al. 2003]. Since *Morus* species are cross-pollinated and mostly dioecious, propagation through seeds does not conserve true-to-types because of its heterozygous nature [Hossain et al. 1992]. The genetic characteristics of superior *Morus* species are maintained by the asexual method (grafting, cuttings, layering, and tissue culture) of selected cultivars [Ma et al. 1996]. However, success rate varies depending on genotypes and the methods used. Grafting and budding are the most widely are used propagation methods, since black mulberry plant are more commercially propagated by this way.

The factors like, temperature, humidity, time of grafting, graft type, pest, disease, water exudate and space between wood and bud stick bark. The walnuts, figs, persimmon and mulberries bleed badly during budding and affect the success of grafting [Güneş and Çekiç 2011, Hartmann et al. 2011, Ahmed et al. 2012, Zenginbal 2015, Zenginbal and Eşitken 2016]. There are some studies conducted the black mulberry

✉ hzenginbal@gmail.com

propagation by various budding and grafting methods on different grafting dates in Turkey [Vural et al. 2008, Güneş and Çekiç 2011, Zenginbal and Eşitken 2016]. The suitability of budding methods, grafting tool and grafting dates in black mulberry propagation has not been studied in details.

The demand for the grafted plant material is increasing day by day in Turkey. The best technique to increase the production of grafted plant material is to adopt asexual propagation. Keeping in view the importance of increasing the demand of grafted plants, the present study was to investigate the effects of budding types, grafting tool for budding and budding dates on graft success, shoot diameter and shoot length of black mulberry.

MATERIALS AND METHODS

Study site

The investigation was carried out at Bolu, Turkey (longitude 40°43'N, latitude 31°33'E, altitude 768 m) for two consecutive years of 2014 and 2015. The rootstocks were grown in the UV polythene bags (5 litres) filled with garden soil, peat and farmyard manure (2 : 1 : 1). Medium soil analyses were as follow: pH 6.72; organic matter 8.12%; total nitrogen content 0.30%; available P_2O_5 60.1 mg kg^{-1}; exchangeable K_2O 466.8 mg kg^{-1}; total organic carbon 4.61%; EC 0.43 mS cm^{-1}; total salt 0.048%; active lime 1.2%; lime ($CaCO_3$) 3.0%.

Plant materials (scion and rootstock)

Two-year old *Morus alba* L. (white mulberry) seedlings having uniform diameter (between 6.0–8.0 mm) were used as rootstocks. Plants of *Morus nigra* L. (black mulberry) were used for scion collection. The scions (one-year old shoots) were selected from healthy 15-year old donor trees on February 1, 2014 and 2015 in Bolu, Turkey. These shoots (30–35 cm long) were disease-free and lignified. Grafting scions were stored between layers of moist paper in polyethylene bags at 4 ±1°C until used. Irrigation, weeding and removal of suckers below graft union were performed regular at one-week interval.

Grafting times and grafting methods

Grafting operations were conducted on March 15, April 1, April 15, May 1 and May 15 of 2014 and 2015. T-budding, inverted T-budding, chip budding, I-budding and patch budding by hand and chip budding with grafting tool were investigated. Chip bud manual grafting tool is produced in Italy by Art Tec Company (Manuel Grafting 3T Art. AR-INN3T) (fig. 1). Buddings were applied as described by Hartmann et al. [2011]. Soft and white silicone plastic tapes were used to wrap the budding. The grafts were done by researcher having extensive experience and about grafting [Zenginbal 2015].

Weather conditions during experiments

Daily mean temperature and relative humidity were scored (one hour's intervals) by mechanical data logger (HOBO U10 Temp/RH data logger) during March 15 to November 15, 2014 and 2015 years.

Investigation of criteria for grafting success

Bud take rate was recorded sixty days after grafting, while sprouting rate was recorded ninety days after grafting. At the end of growing season (November 15), graft shoot length (cm) and diameter (mm) were determined.

Experimental design and statistical analyses

Complete randomized block design was applied with three replications and 20 grafts per replication. The experiment was evaluated as six (budding methods) X five (budding dates) factorial design. Data were examined using SPSS statistical package [SPSS 2011] via license of Ondokuz Mayıs University. Before the ANOVA tests, homogeneity of variances were examined with Levene variance homogeneity test and were found homogeneous (P > 0.05). Normality assumption was examined with Kolmogorov-Smirnov one-sample test and results showed that data has normal distribution, so applying ANOVA was approved. Duncan's test was used for multiple comparisons.

Fig. 1. The methods of budding used in research and grafting processes (a. budding tools, b. preparation of the bud scion, c. preparation of the rootstock, d. the bud scion placement of the detached bud on the rootstock, e. the bud is held in place with white and soft plastic tapes)

RESULTS AND DISCUSSION

Climatic data

Mean daily temperature (°C) and daily relative humidity (%) is given in Figure 2. In the both years of the study, the mean daily temperature and relative humidity were recorded from March 15th, which was the date for the first grafting, to November 15th, which was the date that the data was collected (end of vegetation). In 2014, mean daily temperatures varied from 0.1°C to 25.4°C while the mean daily relative humidity varied from 41.4% to 96.1%. In the second year of the study, the mean daily temperature varied from 0.2°C to 24.2°C while the mean daily relative humidity varied from 35.1% to 96.7%. In both years, temperature began to increase day by day from March 15th, reaching to the highest values between July and August. After August, the average temperature decreased. During both years, daily relative humidity was low in periods which the temperature was high, and high in periods which the temperature was low. Temperature and relative humidity data of the experiment site was compatible with the long term data of Bolu, Turkey [TSMS 2016]. Therefore, it can be said that the experimental years 2014 and 2015 were not extreme in terms of climatic conditions and thus the results obtained in the study can be generalized.

Bud take and sprouting

The data in terms of bud take and sprouting are presented in Table 1. The interaction between methods and the budding dates had significant effect ($P < 0.001$) on bud take and sprouting in both years. The highest graft take rate (88.33% and 85.0%, respectively to years) and sprouting rate (85.0% and 71.67%, respectively to years) were obtained from chip budding by hand performed on May 1, 2014, and 2015. In research, main effects of budding method had significant effect ($P < 0.001$) on but take

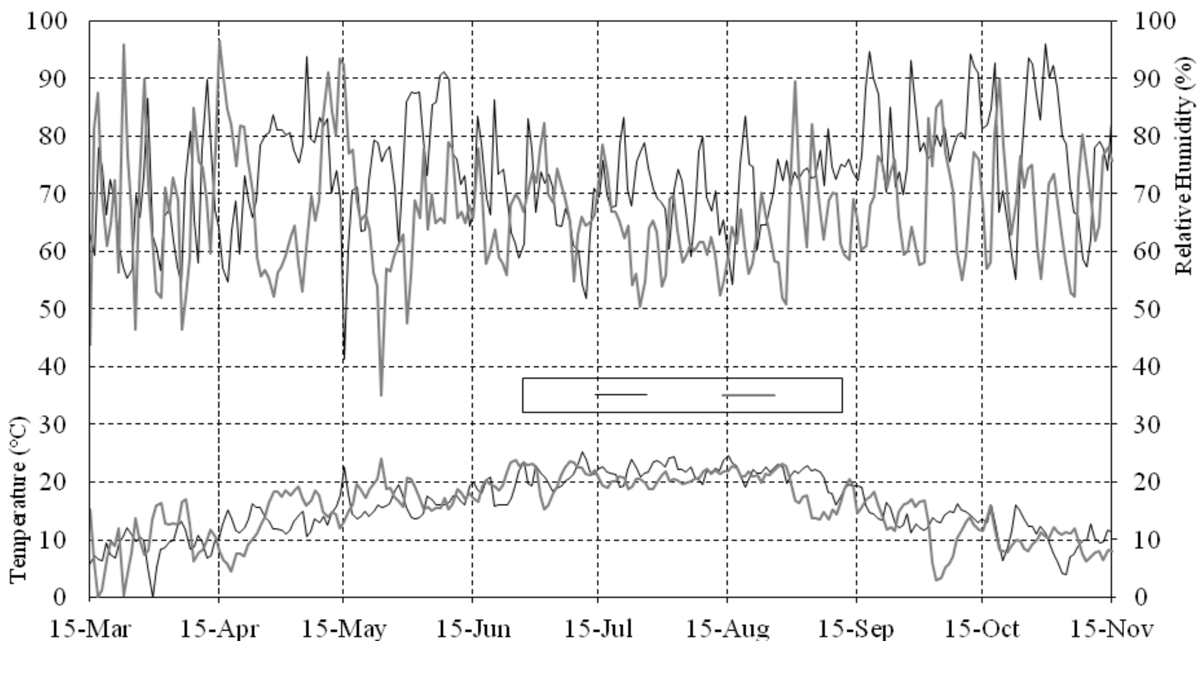

Fig. 2. Average daily temperature and relative humidity values during March 15 to November 15, 2014 and 2015

Table 1. The effect of budding methods and grafting dates on the bud take and bud sprouting of black mulberry

Budding methods	Budding periods	Bud take (%)		Sprouting (%)	
		in 2014	in 2015	in 2014	in 2015
T-budding	March 15	05.00 ±2.50 l	00.00 ±0.00 l	03.33 ±0.00 j	00.00 ±0.00 j
	April 1	21.67 ±1.67 jk	15.00 ±2.89 kl	11.67 ±1.67 ij	06.67 ±1.67 j
	April 15	61.67 ±4.41 d-f	55.00 ±2.89 ef	51.67 ±4.41 de	41.67 ±4.41 ef
	May 1	78.33 ±1.67 a-c	71.67 ±4.41 bc	68.33 ±1.67 b	61.67 ±4.41 b
	May 15	85.00 ± 2.89 ab	71.67 ±7.26 bc	65.00 ±5.00 bc	58.33 ±6.01 bc
Inverted T-budding	March 15	05.00 ±2.50 l	00.00 ±0.00 l	03.33 ±0.00 j	00.00 ±0.00 j
	April 1	18.33 ±1.67 kl	15.00 ±2.50 kl	10.00 ±2.89 ij	06.67 ±1.67 j
	April 15	68.33 ±1.67 c-e	58.33 ±7.26 de	55.00 ±2.89 c-e	43.33 ±8.82 ef
	May 1	75.00 ±5.00 bc	68.33 ±7.26 b-d	66.67 ±6.01 bc	58.33 ±7.26 bc
	May 15	78.33 ±7.26 a-c	65.00 ±5.00 cd	61.67 ±1.67 b-d	55.00 ±5.00 be
Chip budding by hand	March 15	15.00 ±2.89 kl	08.33 ±1.67 kl	08.33 ±1.67 j	03.33 ±1.67 j
	April 1	41.67 ±4.41 hi	38.33 ±6.01 h-j	31.67 ±1.67 fg	28.33 ±4.41 fg
	April 15	71.67 ±4.41 cd	65.00 ±5.00 cd	68.33 ±4.41 b	58.33 ±8.33 bc
	May 1	88.33 ±1.67 a	85.00 ±8.66 a	85.00 ±2.89 a	71.67 ±8.33 a
	May 15	71.67 ±4.41 cd	71.67 ±4.41 bc	65.00 ±5.00 bc	61.67 ±4.41 b
I-budding	March 15	06.67 ±2.50 l	00.00 ±0.00 l	05.00 ±2.50 j	00.00 ±0.00 j
	April 1	21.67 ±1.67 jk	15.00 ±2.50 kl	15.00 ±2.89 h-j	08.33 ±1.67 j
	April 15	58.33 ±6.01 ef	51.67 ±9.28 e-g	48.33 ±7.26 e	38.33 ±6.01 f
	May 1	71.67 ±4.41 cd	61.67 ±7.26 c-e	58.33 ±1.67 b-e	45.00 ±7.64 e
	May 15	68.33 ±1.67 c-e	51.67 ±9.28 e-g	51.67 ±4.41 de	41.67 ±4.41 ef
Patch-budding	March 15	00.00 ±0.00 l	00.00 ±0.00 l	00.00 ±0.00 j	00.00 ±0.00 j
	April 1	15.00 ±2.50 kl	06.67 ±2.50 l	05.00 ±2.50 j	03.33 ±1.67 j
	April 15	41.67 ±4.41 hi	35.00 ±7.64 ij	26.67 ±1.67 fh	25.00 ±5.00 fh
	May 1	51.67 ±4.41 f-h	48.33 ±7.26 gh	35.00 ±5.00 f	35.00 ±5.00 f
	May 15	55.00 ±2.89 fg	51.67 ±9.28 e-g	35.00 ±2.89 f	41.67 ±7.26 ef
Chip budding with tool	March 15	06.67 ±2.50 kl	00.00 ±0.00 l	03.33 ±0.00 j	00.00 ±0.00 j
	April 1	31.67 ±1.67 ij	25.00 ±5.00 jk	15.00 ±2.89 h-j	11.67 ±0.67 ij
	April 15	41.67 ±1.67 hi	35.00 ±2.89 ij	25.00 ±5.00 fh	25.00 ±2.89 fh
	May 1	55.00 ±2.89 fg	48.33 ±7.26 gh	35.00 ±2.89 f	35.00 ±2.89 f
	May 15	45.00 ±5.00 gh	41.67 ±4.41 hi	21.67 ±1.67 g-i	25.00 ±5.00 fh
P value		<0.001	<0.001	<0.001	<0.001

The means ± standard error followed by the same letter were not significantly different (in both years)

Table 2. The effect of budding methods on the bud take and bud sprouting of black mulberry

Budding methods	Bud take (%)		Sprouting (%)	
	in 2014	in 2015	in 2014	in 2015
T-budding	50.33 ±4.34 b	42.67 ±6.32 b	40.00 ±6.82 b	33.67 ±6.55 b
Inverted T-budding	49.00 ±4.20 b	41.33 ±6.26 b	39.33 ±6.58 b	32.67 ±6.84 b
Chip budding by hand	57.67 ±4.33 a	53.67 ±7.75 a	51.67 ±6.84 a	44.67 ±5.64 a
I-budding	45.33 ±4.62 c	36.00 ±3.67 bc	35.67 ±4.43 c	26.67 ±4.43 c
Patch-budding	32.67 ±2.67 d	28.33 ±3.40 c	20.33 ±2.32 d	21.00 ±3.80 d
Chip budding with tool	36.00 ±4.00 d	30.00 ±3.57 c	20.00 ±2.12 d	19.33 ±2.88 d
P value	<0.001	<0.001	<0.001	<0.001

The means ± standard error followed by the same letter were not significantly different (in both years)

Table 3. The effect of grafting dates on the bud take and bud sprouting of black mulberry (in both years)

Budding periods	Bud take (%)		Sprouting (%)	
	in 2014	in 2015	in 2014	in 2015
March 15	06.38 ±1.65 d	01.38 ±1.67 d	04.58 ±1.25 c	00.55 ±0.00 c
April 1	25.00 ±2.50 c	19.17 ±2.91 c	14.72 ±2.52 c	10.83 ±2.52 c
April 15	57.22 ±3.70 b	50.00 ±3.73 b	45.83 ±3.56 b	38.61 ±3.52 b
May 1	70.00 ±4.50 a	63.89 ±3.99 a	58.05 ±3.72 a	51.11 ±3.97 a
May 15	67.22 ±4.25 ab	58.89 ±3.85 ab	50.00 ±3.73 b	47.22 ±3.80 a
P value	<0.001	<0.001	<0.001	<0.001

The means ± standard error followed by the same letter were not significantly different (in both years)

and sprouting in 2014 and 2015 (tab. 2). Chip budding by hand was obtained highest bud take rate (57.67% and 53.67%, respectively to years) and graft sprouting rate (51.67% and 44.67%, respectively to years). Chip budding with tool and patch budding methods were recorded lowest results for these parameters in both years. As shown in Table 3, main effects of budding dates had significant effect ($P < 0.001$) on bud take and sprouting in both years. Budding dates on May 1 and May 15, 2014 and 2015 gave the highest rate of bud take and sprouting. The lowest results were obtained on March 1 and April 1 in both years.

Chip budding by hand was found to be successful, patch budding and chip budding with tool were found to be unsuccessful to black mulberry plant production. The success of chip budding by hand may be attributed to high level of callus formation around the grafting location. Likewise, several researchers [Çelik et al. 2006, Zenginbal and Dolgun 2014, Zenginbal 2015, Zenginbal and Eşitken 2016] reported that callus formation was higher and faster for chip budding. In addition, preparation of graft scion also affected the graft success [Hartmann et al. 2011]. Graft scions (single bud) were prepared with wood tissue in chip budding and without wood tissue in T, inverted T, I and patch budding. As a result of the research, T, inverted T, I and patch budding methods were obtained lower graft success. The reason for this may be due to tissue damages on bud while preparing graft scion. Likewise, pith tissue is wide and stem tissues are friable in mulberry. Therefore, cortex is separated from wood tissue of grafting resulting in enormous damage in graft scion bud as observed by Hartmann et al. [2011]. It was also observed that rootstock could not soak water sufficiently on March 15, April 1 and April 15 when grafts were performed. This phenomenon affected the success rate of T, inverted T, I and patch budding. So, success depends on easily separation of bark from wood tissue which is possible effective water soaking of plant tissues. But, there is no need water soaking for chip budding by hand and with tool applications. If water presence is weak between bark and wood tissues, as observed in the present study, barks have cracked causing the expansion of wounded tissue and not cohering of graft. Hartmann et al. [2011] and Zenginbal [2015] reported that excessive cutting surface affected graft success negatively. Chip budding with tool produced lower results. This is probably due to tissue damages by budding with manual tools. Our findings support the results of Çelik [2000] and Zenginbal [2015]. Similar results were also obtained by Çelik and Boz [2003] in grapevine, Çelik et al. [2006] in kiwifruit and Zenginbal [2015] in persimmon. As result of research, the highest bud take and sprouting rate were obtained on May 1, closely followed by May 15, while lowest results for these parameters were obtained on March 15 and April 1. The lowest percentage of bud take and sprouting obtained on March 15 and April 1 grafting in comparison to the May 1 and May 15 grafting might due to the fact that on March 15 and April 1 growth of tissue in dormant period and loses their tolerance to injury. Besides, the low success of grafting on March 15 and April 1 might have caused due to inadequate flow of cell sap. This finding is in agreement with the results of Güneş and Çekiç [2011] and Zenginbal and Eşitken [2016] in black mulberry. The reason of lower success of budding were performed on May 15 comparison to the May 1 might due to excess bleeding (milk secretion) in the rootstock. Hartman et al. [2011] and Zenginbal [2015] also indicated that the amount of bleeding in the rootstocks affect the success of grafting. Özkaya Erkaleli and Dalkılıç [2016] in their study on phenology of black mulberry varieties in Uşak, Turkey reported that the first bud burst begin on middle of April, the bud sprouting occurred from April 17 to April 21, while the full flowering took place from May 5 to May 9. This is a good time for grafting as growth hormones are concentrated in the buds and these are effective enough in inducing differentiation of vascular elements in the tissues of the graft [Hartmann et al. 2011]. After April 15 is the time when the cold season ends and the rainy season begins in Bolu. This transition from cold to rainy season is the optimum time for grafting because meristematic activity is starting and the scion-rootstock union is established quickly [Leakey and Newton 1994]. The full flowering stage of the black mulberry between May 5 and May 9 is the most intense period in terms of water content, so this finding supports our hypothesis. Such a wide variation of bud take and sprouting observed in different grafting periods may possibly be attributed due to wide variation in temperature and relative humidity during the period of study. Favourable temperature and relative humidity helps in production of more callus tissues in the area grafting [Hartmann et al. 2011]. These factors influence the bud take and sprouting. Güneş and Çekiç [2011] reported suitable temperature for successful black mulberry budding unity should be at least 25°C to 30°C. Higher daily mean temperature occurring from May 1 to May 15 (in both years) (fig. 2) might have played an important role in early contact of cambium layers of rootstock and scion resulting in early callus formation and more success percent of grafts. During May 1 and May 15, higher percent of relative humidity (fig. 2) also helped in production of more callus tissues in the grafts [Zenginbal 2015]. Similar results were also obtained by Vural et al. [2008] and Güneş and Çekiç [2011] in black mulberry.

Shoot length and diameter

The graft shoot length and diameter of black mulberry grafting are summarized in Table 4. The binary interaction (budding method × budding dates) had significant effect ($P < 0.001$) on graft shoot length and diameter in both years. The highest graft shoot lengths (between 38.40 cm and 41.33 cm) were recorded with T, inverted T and chip budding performed on April 15 and May 1, 2014. In 2015, the

Table 4. The effect of budding methods and budding dates on the graft shoot length and diameter of black mulberry

Budding methods	Budding periods	Shoot length (cm)		Shoot diameter (mm)	
		in 2014	in 2015	in 2014	in 2015
T-budding	March 15	08.75 ±0.00 n	00.00 ±0.00 l	1.52 ±0.00 m	0.00 ±0.00 l
	April 1	36.90 ±0.39 b-f	33.64 ±1.69 c-f	5.69 ±0.12 b-g	5.68 ±0.22 b-f
	April 15	39.24 ±1.47 a-c	36.89 ±1.17 b-e	5.80 ±0.43 b-e	5.76 ±0.44 b-e
	May 1	39.38 ±1.18 a-c	39.30 ±2.07 a	5.74 ±0.27 b-f	6.08 ±0.19 ab
	May 15	35.89 ±1.30 b-f	33.42 ±1.64 c-f	5.33 ±0.09 d-h	5.32 ±0.29 c-h
Inverted T-budding	March 15	06.24 ±0.00 n	00.00 ±0.00 l	1.44 ±0.00 m	0.00 ±0.00 l
	April 1	37.85 ±0.54 b-e	23.84 ±1.99 h-j	6.02 ±0.10 ab	4.13 ±0.20 jk
	April 15	41.33 ±0.67 a	39.68 ±1.13 a	6.26 ±0.06 a	6.26 ±0.24 a
	May 1	39.86 ±0.74 ab	38.28 ±1.45 ab	5.99 ±0.12 ab	6.14 ±0.18 a
	May 15	36.98 ±1.10 b-f	37.60 ±1.38 b-c	5.85 ±0.13 b-d	6.05 ±0.14 ab
Chip budding by hand	March 15	33.69 ±0.38 e-h	10.07 ±0.00 k	4.82 ±0.04 h-k	1.71 ±0.00 kl
	April 1	35.65 ±0.55 b-f	32.77 ±1.52 d-g	5.38 ±0.19 c-h	5.28 ±0.28 d-h
	April 15	38.67 ±1.52 a-d	37.43 ±1.04 b-c	5.63 ±0.26 b-g	5.59 ±0.40 b-g
	May 1	38.40 ±1.58 a-d	35.61 ±1.75 b-e	5.24 ±0.26 e-h	5.59 ±0.27 b-g
	May 15	35.07 ±1.35 c-g	32.19 ±1.62 d-g	5.20 ±0.14 f-h	5.13 ±0.20 f-h
I-budding	March 15	14.32 ±1.25 m	00.00 ±0.00 l	3.26 ±0.21 h-k	0.00 ±0.00 l
	April 1	34.38 ±1.01 d-h	23.11 ±2.11 h-j	5.95 ±0.14 b-c	4.24 ±0.20 i-k
	April 15	36.31 ±1.19 b-f	35.46 ±1.20 b-e	5.89 ±0.12 b-d	5.98 ±0.21 b-c
	May 1	36.68 ±1.96 b-f	36.14 ±2.91 b-e	5.90 ±0.10 b-d	6.07 ±0.23 ab
	May 15	33.09 ±2.47 f-i	33.69 ±2.41 c-f	5.35 ±0.07 d-h	5.79 ±0.08 b-d
Patch-budding	March 15	00.00 ±0.00 n	00.00 ±0.00 l	0.00 ±0.00 m	0.00 ±0.00 l
	April 1	12.87 ±0.95 m	07.41 ±0.00 kl	2.92 ±0.07 kl	1.49 ±0.00 kl
	April 15	25.59 ±1.43 kl	27.89 ±1.99 g-j	5.14 ±0.24 g-i	5.52 ±0.11 b-g
	May 1	29.51 ±1.95 ij	30.32 ±1.56 e-i	5.71 ±0.02 b-g	5.55 ±0.40 b-g
	May 15	31.44 ±1.35 g-j	29.67 ±1.25 f-i	5.89 ±0.11 b-d	5.76 ±0.54 b-e
Chip budding with tool	March 15	14.76 ±0.01 lm	00.00 ±0.00 l	2.82 ±0.01 l	0.00 ±0.00 l
	April 1	28.20 ±1.11 jk	28.55 ±1.30 f-j	4.59 ±0.08 i-l	4.89 ±0.14 h-j
	April 15	35.09 ±0.92 c-g	30.79 ±1.32 e-h	5.04 ±0.22 h-j	5.15 ±0.24 f-h
	May 1	35.52 ±1.04 c-g	34.33 ±2.06 c-f	4.91 ±0.11 h-k	5.12 ±0.19 f-h
	May 15	30.86 ±0.30 h-j	30.66 ±1.11 e-h	4.51 ±0.07 j-l	5.02 ±0.12 g-i
P value		<0.001	<0.001	<0.001	<0.001

The means ± standard error followed by the same letter were not significantly different (in both years)

Table 5. The effect of budding methods on the graft shoot length and diameter of black mulberry

Budding methods	Shoot length (cm)		Shoot diameter (mm)	
	in 2014	in 2015	in 2014	in 2015
T-budding	32.03 ±0.92 b	35.81 ±1.02 b	4.81 ±0.18 b	5.71 ±0.15 bc
Inverted T-budding	32.45 ±1.13 b	38.02 ±0.71 a	5.11 ±0.16 ab	6.16 ±0.08 a
Chip budding by hand	36.30 ±1.22 a	34.50 ±0.91 b	5.26 ±0.22 a	5.40 ±0.14 cd
I-budding	30.96 ±0.82 b	35.07 ±0.99 b	5.27 ±0.14 a	6.02 ±0.10 ab
Patch-budding	19.88 ±0.52 d	29.29 ±0.89 c	3.93 ±0.16 d	5.61 ±0.20 c
Chip budding with tool	28.89 ±0.42 bc	31.08 ±0.89 c	4.38 ±0.17 c	5.05 ±0.08 d
P value	<0.001	<0.001	<0.001	<0.001

The means ± standard error followed by the same letter were not significantly different (in both years)

Table 6. The effect of budding dates on the graft shoot length and diameter of black mulberry

Budding periods	Shoot length (cm)		Shoot diameter (mm)	
	in 2014	in 2015	in 2014	in 2015
March 15	12.96 ±0.98 c	01.68 ±0.00 c	3.96 ±0.12	0.29 ±0.00
April 1	30.97 ±0.82 b	24.92 ±0.92 b	5.09 ±0.19	4.28 ±0.17
April 15	36.04 ±0.97 a	34.69 ±1.09 ab	5.63 ±0.38	5.71 ±0.43
May 1	36.56 ±1.06 a	35.66 ±0.99 a	5.58 ±0.16	5.76 ±0.13
May 15	33.89 ±1.06 b	32.87 ±0.83 b	5.36 ±0.28	5.51 ±0.23
P value	<0.001	<0.001	>0.05	>0.05

The means ± standard error followed by the same letter were not significantly different (in both years)

highest results (between 38.28 cm and 39.68 cm) were recorded with T budding performed on May 1 and inverted T budding performed on April 15 and May 1. The highest graft shoot diameters (6.02 mm, 6.26 mm and 5.99 mm, respectively) were recorded with inverted T budding performed on April 1, April 15 and May 1, 2014. In 2015, the highest graft shoot diameters (between 6.05 mm and 6.26 mm) were recorded with T budding performed on May 1, inverted T budding performed on April 15, May 1 and May 15, and I budding performed on May 1. As shown in Table 5, main effects of budding method had significant effect (P < 0.001) on graft shoot length and diameter in both years. In 2014, chip budding by hand was recorded highest graft shoot length (36.30 cm), while in 2015, inverted T budding was recorded highest graft shoot length (38.02 cm). Patch budding method was recorded lowest results for these parameters in both years. The highest graft shoot diameters (5.11 mm, 5.26 mm and 5.27 mm, respectively) were recorded with inverted T, T and I budding in 2014, while in 2015, the highest graft shoot diameters (6.16 mm and 6.02, respectively) were recorded with inverted T and I budding. In research, main effects of budding dates had significant effect (P < 0.001) on graft shoot length and insignificant effect (P > 0.05) on graft shoot diameter in both years. Budding dates on April 15 and May 1, 2014 and 2015 gave the highest result of graft shoot length and diameter. The lowest results were obtained on March 15 in both years (tab. 6).

In general, chip budding by hand method was found to be superior to other buddings methods. The quick and strong union formation, greater uptake of water and nutrients, and longer growing period may account for higher growth of chip budded plants. Similar results were also obtained maximum length and diameter of graft shoots with chip budding by Çelik et al. [2006] in kiwifruit, Zenginbal and Dolgun [2014] in apple, Zenginbal [2015] in persimmon and Zenginbal and Eşitken [2016] in black mulberry. As result of research, the length and diameter of graft shoots were recorded maximum on May 1 and April 15 after grafting, closely followed by May 15 and April 1 whereas, it was recorded minimum in March 15. The higher growth of plants grafted on May 1 and April 15 may be attributed to early union formation and greater duration of growing period as compared to grafting on May 15. In addition, the quick and strong union formation, better nutrient uptake and ample growing period might have caused for higher plant growth and more length and diameter of graft shoots on May 1 and April 15. These results are partially in harmony with the finding of Zenginbal et al. [2007] in kiwifruit and Zenginbal [2015] in persimmon.

CONCLUSION

According to the results of this study, of all parameters the best method of budding was chip budding by hand, followed by T and inverted T-budding for black mulberry nursery plant production. The chip budding with manual tool and patch budding produced the lowest results in all budding dates. The best date of grafting was obtained on May 1, followed on May 15.

REFERENCES

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 J. Agric. Sci., 82(12), 14–18.

Anis, M., Faisal, M., Singh, S.K. (2003). Micropropagation of mulberry (*Morus alba* L.) through in vitro culture of shoot tip and nodal explants. Plant Tissue Cult., 13(1), 47–51.

Çelik, H. (2000). The effects of different grafting methods applied by manual grafting units on grafting success in grapevines. Turk. J. Agric. For., 24, 499–504.

Çelik, H., Boz, Y. (2003). Hand manual grafting units for grapevine propagation. Centenary Jubilee of the IVE-Pleven, Celebration and Scientific Workshop, 13–14 June 2002, SPS Print, 150–159.

Çelik, H., Zenginbal, H., Özcan, M. (2006). Effect of budding performed by hand and with manual grafting unit on kiwifruit propagation in the field. Hort. Sci. (Prague), 33(2), 57–60.

Elmacı, Y., Altuğ, T. (2002). Flavour evaluation of three black mulberry (*Morus nigra*) cultivars using GC/MS, chemical and sensory data. J. Sci. Food Agric., 8, 632–635.

Güneş, M., Çekiç, Ç. (2011). Effects of various rootstocks, budding times and techniques on budding success of black mulberry. Propag. Ornam. Plants, 11(1), 44–46.

Hartmann, H.T., Kester, D.E., Davies, Jr., F.T., Geneve, R.L. (2011). Plant propagation: principles and practices. 8th ed. Prentice Hall, Upper Saddle River, NJ, 915 p.

Hossain, M., Rahman, S.M., Zaman, A., Joarder, O.I., Islam, R. (1992). Micropropagation of *Morus laevigata* Wall. from mature trees. Plant Cell Rep., 11, 522–524.

Koyuncu, F., (2004). Morphological and agronomical characterization of native black mulberry (*Morus nigra* L.) in Sütçüler, Turkey. Plant Genet. Res. Newsl., 138, 32–35.

Leakey, R.R.B., Newton, A.C. (1994). Domestication of tropical trees for timber and non-timberproducts. MAB Digest 17, UNESCO, Paris 94 p.

Lu, M.-C. (2002). Micropropagation of *Morus latifolia* poilet using axillary buds from mature trees. Sci. Hort., 96, 329–341.

Ma, F., Guo, C., Liu, Y., Li, M., Ma, T., Mei, L., Hsiao, A.I. (1996). *In vitro* shoot-apex grafting of mulberry (*Morus alba* L.). HortScience, 31(3), 460–462.

Özkaya Erkaleli, Z., Dalkılıç, Z. (2016). Determination of morphological, phenological and pomological characterization of black mulberry (*Morus nigra* L.) grown in Ulubey Vicinity, Usak province, 13(1), 89–106 (in Turkish).

Özrenk, K., Gazioğlu Şensoy, R.I., Erdinç, C., Güleryuz, M., Aykanat, A. (2010). Molecular characterization of mulberry germplasm from Eastern Anatolia. Afr. J. Biotechnol., 9(1), 1–6.

SPSS, IBM Corp. (2011). IBM SPSS Statistics for Windows, Version 20.0. IBM Corp, Armonk, NY.

TSMS (2016). Turkish State Meteorological Service. Official Statistics (Statistical Database of Bolu, Turkey).

Vural, U., Dumanoğlu, H., Erdoğan, V. (2008). Effect of grafting/budding techniques and time on propagation of black mulberry (*Morus nigra* L.) in cold temperate zones. Propag. Ornam. Plants, 8(2), 55–58.

Zenginbal, H., Özcan, M., Haznedar, A., Demir, T. (2007). Comparisons of methods and time of budding in kiwifruit (*Actinidia deliciosa*, A. Chev.). IJNES, 1, 23–28.

Zenginbal, H., Dolgun, O. (2014). Determining of suitable graft method for apple propagation in cool climatic and high altitude conditions. Int. J. Agric. For. Fish., 2, 53–59.

Zenginbal, H. (2015). Effect of budding methods and grafting periods on production of persimmon sapling in nursery conditions. Propag. Ornam. Plants, 15(1), 3–9.

Zenginbal, H., Eşitken, A. (2016). Effects of the application of various substances and grafting methods on the grafting success and growth of black mulberry (*Morus nigra* L.). Acta Sci. Pol. Hortorum Cultus, 15(4), 99–109.