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Determining of suitable graft method for apple propagation in cool climatic and high altitude conditions

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Abstract

The objective of this study was to determine the most suitable budding methods in different apple cultivars. In this study, MM 109 was used as rootstock under field conditions. Granny Smith, Red Chief, Golden Delicious and Mondial Gala apple cultivars were used as grafting scions. The chip and T budding were grafting methods. All budding operations were done on the 25th April in 2012. Bud taking rate, bud sprouting rate, graft shoot diameter, shoot length and graft combinations were determined in this study. Bud taking and sprouting success resulted in sufficient values for all cultivars and grafting types. Also, satisfactory values for graft diameter and length shoot were obtained. The conclusion was that chip buddings were superior to T buddings; and Mondial Gala was superior to other cultivars in terms of graft shoot quality. Successful results were obtained in all grafting combinations of chip budding. The success could be attributed in chip budding that it caused the less necrotic tissue in blanking between rootstock and scion then better cambial continuity and vascular integration occurred.

Keywords

MalusCommunis L., Budding Methods, Graft Formation

1. Introduction

Apple is a fruit growing from cold places like Siberia and China to warm places such as Colombia and Indonesia. Leading apple producing countries are China, USA, Iran, Turkey, Russia, Italy, India, France, Chili, Argentina, Brasilia and Poland (Atay et al. 2010; Anon. 2013a).

Apple is produced almost in every region of Turkey. Bolu, where the study was conducted has a share of 2.78% in Turkey, is the highest producer in the Western Black Sea Region of Turkey (Anon. 2013b).

For apple production, Turkey is the fourth country in the world, but the yield is very low (Anon. 2013a, b). Possible causes for this situation are insufficient field applications, high quality saplings not be used and other deficiencies for sapling production and distribution. In order to go further in the country's apple growing, saplings of varieties of quality and high market value needs to be produced (Güleryüz and Ertürk2000).

The apple is reproduced with grafting. Grafting success has been affected by many factors such as plant species, rootstock-scion combination, grafting method, and ecological conditions (temperature, humidity, oxygen etc.) during grafting period (Hartmann et al. 2002).

The effect of cultivars on grafting success in apple sampling production has varied. Köksal and Kantarcı (1985), while studying T budding on apple seedling rootstocks inoculated different apple cultivars during June 18th- 24th and reported grafting success ratio as 70.4%,%91.1, and 65.9%for Amasya, Golden Delicious and Starking cultivars, respectively. Kopuzoğlu and Odabaş (1992), while applying indoor grafting onto seedling groot stocks inoculated Starkspur Golden Delicious, Starking

Delicious, Golden Delicious and Amasya apple cultivars and reported that grafting success ratio was 97.5%, 82.5%, 70.0%, and100%, respectively. Kadan and Yarılgaç (2005), in a study on apple, performed the T budding onto apple seedling rootstock grafted Starking Delicious and Golden Delicious apple cultivars during the dormant season. No differences were detected among cultivars for bud taking, sprouting, and shoot length.

Another factor affecting grafting success is grafting type. The most common type is budding although both budding and grafting are used. There are different kinds of budding types such as T budding, inverted T budding, I budding, patch budding, chip budding, and etc. (Köksal and Kantarcı 1991; Hartmann et al. 2002). Hartmann et al. (2002) reported that T budding was used generally in reproduction of fruit trees and chip buddings were not preferred since they were not simpler and faster in application. In contrast, Kviklis (1986) reported that chip budding was more practical and grafting success was higher. He also reported that chip budding was possible to make 363 grafting per hour and this value is significantly higher than T budding (234 grafting per hour).In another study, Howard et al. (1974), applied different methods of budding (T, inverted T and chip budding) on an old apple seedling rootstock. According to the results, chip budding increased sapling growth, the lateral branch number and length significantly. Moreover, Alibert and Masseron (1976) reported that chip budding produced more successful results than T budding for bud taking and sampling height. Stoyan (1984) and Czarneck (1990) reported that samplings grafted by chip budding rowed more robustly and produced more successful results than conventional T budding.

On the other hand, rootstock may be cause to incompatibility during the graft formation. Grafting is a troublesome process and requires a long time to observe grafting success and this situation can cause losing of more money, time and effort. Studying on the sections, prepared from the graft samples, gives us opportunity to assess the development of callus tissue, the position of necrotic layers, cambial differentiating, cambial continuity and the development of vascular tissues. In this regard, histological evaluations on graft sections allow us to get information about first information about compatibility or incompatibility of combinations in a short time (Wertheim 1990; Knowleds et al. 1994; Vachun1995; Gryzb, Sitarek 1998; Kankaya et al.1999).

First response of plant tissues to grafting is callus development in scion and rootstock in two weeks (Errea et al. 1994). Integration of scion and rootstock is depends on callus induction. Callus tissue, rootstock young xylem and xylem extract ray cells; stock of the cambium and the secondary shell cells is formed (Mosse 1962; Dolgun et al. 2008; Polat et al. 2010).

This study was conducted to determine the grafting success and sapling development in four apple cultivars (Granny Smith, Red Chief, Golden Delicious, Mondial Gala) grafted by chip and T budding methods onto MM 109 rootstock in Bolu. Also, graft union formation in relation to compatibility/incompatibility growing features among different apple cultivars described.

2. Material and Method

The study was conducted in experimental fields of Abant İzzet Baysal University, Bolu Vocational College (40° 43' NLat, 31°33'Long., and 768 m elevation)in 2012. Graft samples were investigated in Adnan Menderes University, Sultanhisar Vocational College.

The two years old MM 109 having 6.0-10.0 mm diameters were used as rootstock. Rootstocks were planted in UV supported polyethylene pots filled with special medium (1:1:1garden soil: peat: manure).The growing medium was salt-free, poor in lime, rich in organic matter and humus, very rich in P and K. Medium soil analyses were the following:

pH: 7.58; organic matter: 8.40%; total nitrogen: 0.04%; availableP_2O_5: 364.2 kg/d; available K_2O: 784.8 kg/d; total organic carbon: 4.8; EC: 0.48 ms/cm; total salt: 0.021%; lime ($CaCO_3$): 3.0%; active lime: 1.5%.

Scion woods of the cvs. Granny Smith, Red Chief, Golden Delicious, Mondial Gala were selected in previous winter from vigorous productive plants grown in the apple orchard in Seben region of Bolu. They were packed in dumpy sawdust and stored at 4 °C till the day for the study.

All budding operations were done on the 25th April2012. Chip and T budding kinds of grafting were tried. White and soft plastic tapes were used for wrapping. Cultural practices such as irrigation, weeding and removal of sucker were applied at regular intervals. After 45 days than grafting, bud and sprouting were analyzed during vegetation period, at intervals of 30 days in terms of the bud explosion growth development of the shoot length and diameter values. Furthermore, grafting combinations samples (3graft/cultivar) were collected and placed in alcohol solution of 70% at intervals of 30 days until the end of vegetation period. Transverse sections (20-30µm) were cut with a rotary microtome. All sections were examined under microscope (Mic D),than microphotograph of sections is taken without coloring. The relative humidity and temperature were also recorded.

Experimental design was a randomized complete block with three replications, each including 10 plants. Data were analyzed by MSTAT-C (Russell D. Freed, Crop and Soil Sciences Department, Michigan State University). Data expressed as percentage (bud take, sprouting) were arc-sin$\sqrt{x}$ transformed. Lettering in the table of values was made after data retransformed, in the table was given in parenthesis. Differences among mean values were tested by Duncan's Multiple Range in MSTAT. The differences were explained at 5% as important and 1% as very important.

3. Results and Discussion

The changes in daily temperature (°C) and relative humidity (%) of experimental area were in Fig 1.From April 24 to June 15 which is the most important period for grafting, the average temperatures were13 to 20 °C. Temperature fluctuated after June 15 and reached the maximum in middle of July. After August, the average temperature decreased. Relative humidity varied with 43-98%. The climatic data were in accordance with the long term average of Bolu (Anon. 2013c), and indicating that 2012 was an average year. Thus, generalization from the study seemed possible.

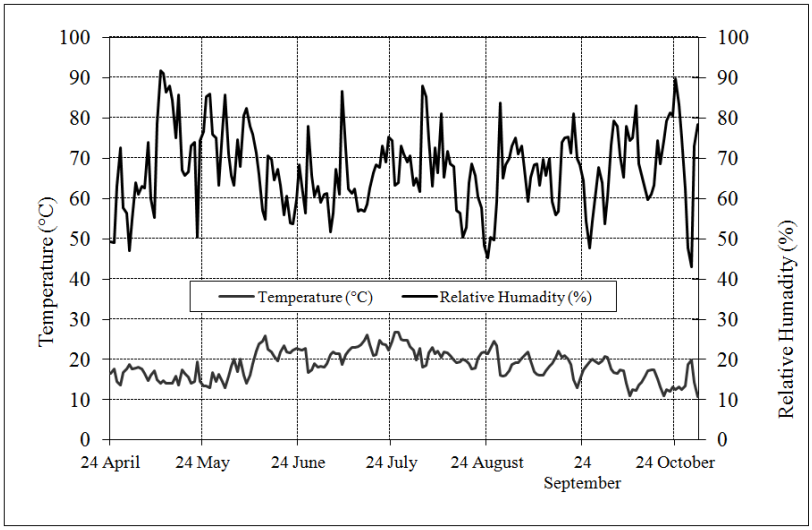

Fig. 1. *Changing of mean temperature and relative humidity during the days after grafting*

Data for apple cultivars grafted by T and Chip budding onto MM 109 apple rootstock are shown in Table 1. All tried combinations had 100% bud take rate. The sprouting rate was significant applications of budding methods ($P<0.01$), apple cultivars ($P<0.05$) and apple cultivar x budding methods interaction ($P<0.05$). The highest sprouting rates (100%) was obtained from all cultivars, grafted by chip budding and from Golden Delicious cultivar, grafted by T budding. The lowest sprouting rate (76.7%) was obtained from Mondial Gala cultivar, grafted by T budding. In terms of shoot length, significant differences were detected between cultivars and grafting methods ($P<0.01$), cultivars x grafting methods interaction was also found to be significant ($P<0.05$) fort this parameter. The highest shoot length (66.0 cm) was obtained from Mondial Gala cultivar, grafted by chip budding methods. The lowest shoot length (42.3 cm) was obtained from Red Chief cultivar, grafted by T budding methods. Differences in shoot diameter of cultivars and cultivar x grafting type interaction were found to be significant ($P<0.05$). Graft shoot diameter was maximum (7.35 mm) in Mondial Gala cv, grafted by chip budding and minimum (5.74 mm) in Golden Delicious cv, grafted with T budding.

Analyzing the monthly development length of shoots in combination grafted onto MM 109 rootstock (Fig 2),an evident shoot development was observed for all combination after June 12. Generally, shoot length increased linearly until August 12 and then stated to slow down for all cultivars. Specially, in Mondial Gala cultivar shoot length increased linearly until October 12, and then evidently slowed in all combinations, finally stopped in November 12.As shown in Fig 3, shoot diameter development started to increase after June 12 in all combinations. Shoot diameter development increased normally until August 12 and then speedy in Golden Delicious cultivar. Shoot diameter development continued to increase up to October 12 and stopped in November 12.

Slides from grafting combinations were screened by microscope and callus induction which was sufficient to integrate rootstock and scion detected in 30 days old age samples. It was observed that cambial continuity and vascular tissues occurred in 60 days samples. As shown in Fig 4 and 5, strong integration was also observed between rootstock and scion in 90-day samples. The present results indicated that chip budding was more suitable method than T budding for apple sapling reproduction in cold climatic conditions. Because formation of necrotic tissue and wounding were less during the chip budding process, heal more quickly realized than T budding. In the chip budding, as rootstock and scion fits very well each other and there is no gap between them, formation of callus is less and fills the gaps fully. Therefore, new occurred cambium is quite smoothly. As shown in graphs (Fig 2 and 3), shoot development and diameter increased evidently at the end of June and at the beginning of July when vascular tissue induction increased. In the T budding, due to weak contact between the scion and rootstock, gaps between them is much. Thus, amount of callus is much and rootstock - scion cambium is not fully reciprocal. Moreover, callus was occurred in stacks and stacks of necrotic tissue were occurred in the budding. Necrotic tissue stacks were not absorbed in the callus. As a result of this, the link between rootstock and scion is provided.

Table 1. *The effect of chip and T budding methods on bud take, sprouting and growth of grafts in different apple cultivars*

	Apple Cultivars	T Budding	Chip Budding	Mean
	Granny Smith	100.0[1] (90.0)[2]	100.0 (90.0)	100.0 (90.0)
	Red Chief	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)
Bud Take (%)	Golden Delicious	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)
	Mondial Gala	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)
	Mean	100.0	100.0	100.0
	Granny Smith	90,0 (78.9) a	100,0 (90.0) a	95.0 (84.5) ab
	Red Chief	80.0 (63.9) b	100.0 (90.0) a	90.0 (77.0) b
Sprouting (%)	Golden Delicious	100.0 (90.0) a	100.0 (90.0) a	100.0 (90.0) a
	Mondial Gala	76.7 (61.2) b	100.0 (90.0) a	88.3 (75.6) b
	Mean	86.67 b**	100.0 a**	93.34
	LSD5% (cultivar x budding method): 12.96		LSD5% (cultivar): 9.17	
	Granny Smith	67.7 a	62.0 ab	64.8 a
	Red Chief	42.3 e	50.3 cd	46.3 b
Shoot Length (cm)	Golden Delicious	44.0 de	63.3 ab	53.7 b
	Mondial Gala	57.0 bc	66.0 a	61.5 a
	Mean	52.8 b**	60.4 a**	56.6
	LSD5% (cultivar x budding method): 7.64		LSD1% (cultivar): 7.50	
	Granny Smith	7.15 ab	6.60 ac	6.88 a
	Red Chief	6.38 bd	6.08 cd	6.23 b
Shoot Diameter (mm)	Golden Delicious	5.74 d	6.23 cd	5.99 b
	Mondial Gala	6.47 bd	7.35 a	6.91 a
	Mean	6.43	6.57	6.50
	LSD5% (cultivar x budding method): 0.72		LSD5% (cultivar): 0.51	

[1] Original data
[2] Transformed data
** Values not associated with the same letter are significantly different (P<.001)

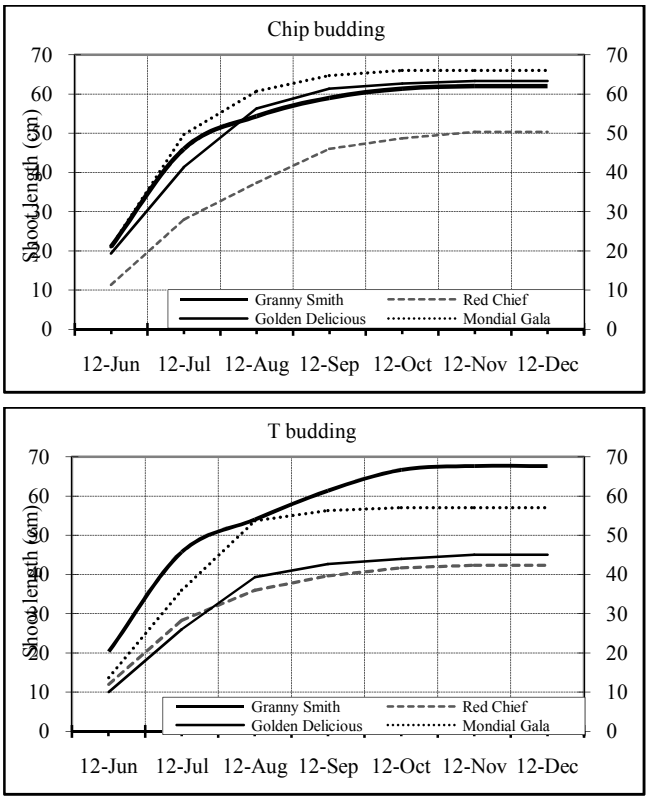

Fig.2. *The development of the shoot length at intervals of 30 days in different Apple cultivars*

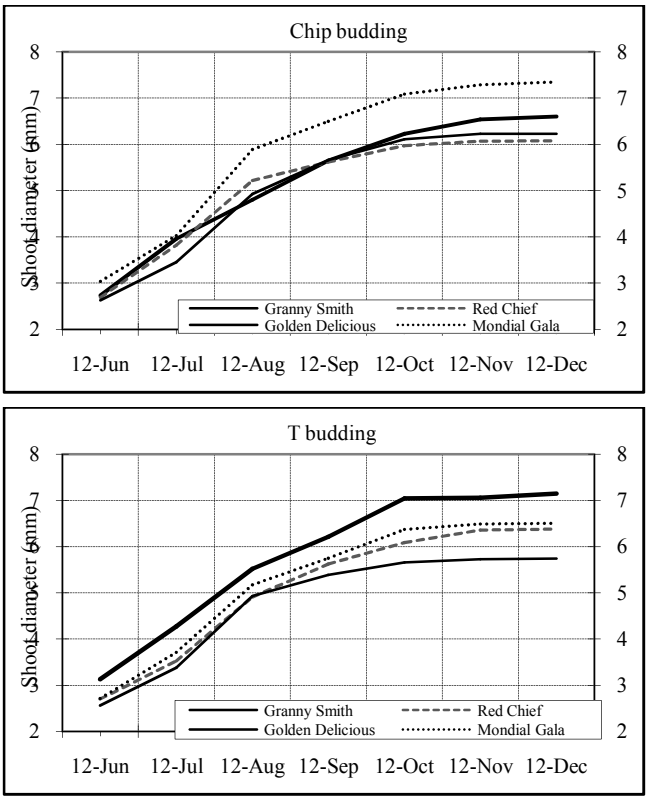

Fig.3. *The development of the shoot diameter at intervals of 30 days in different Apple cultivars*

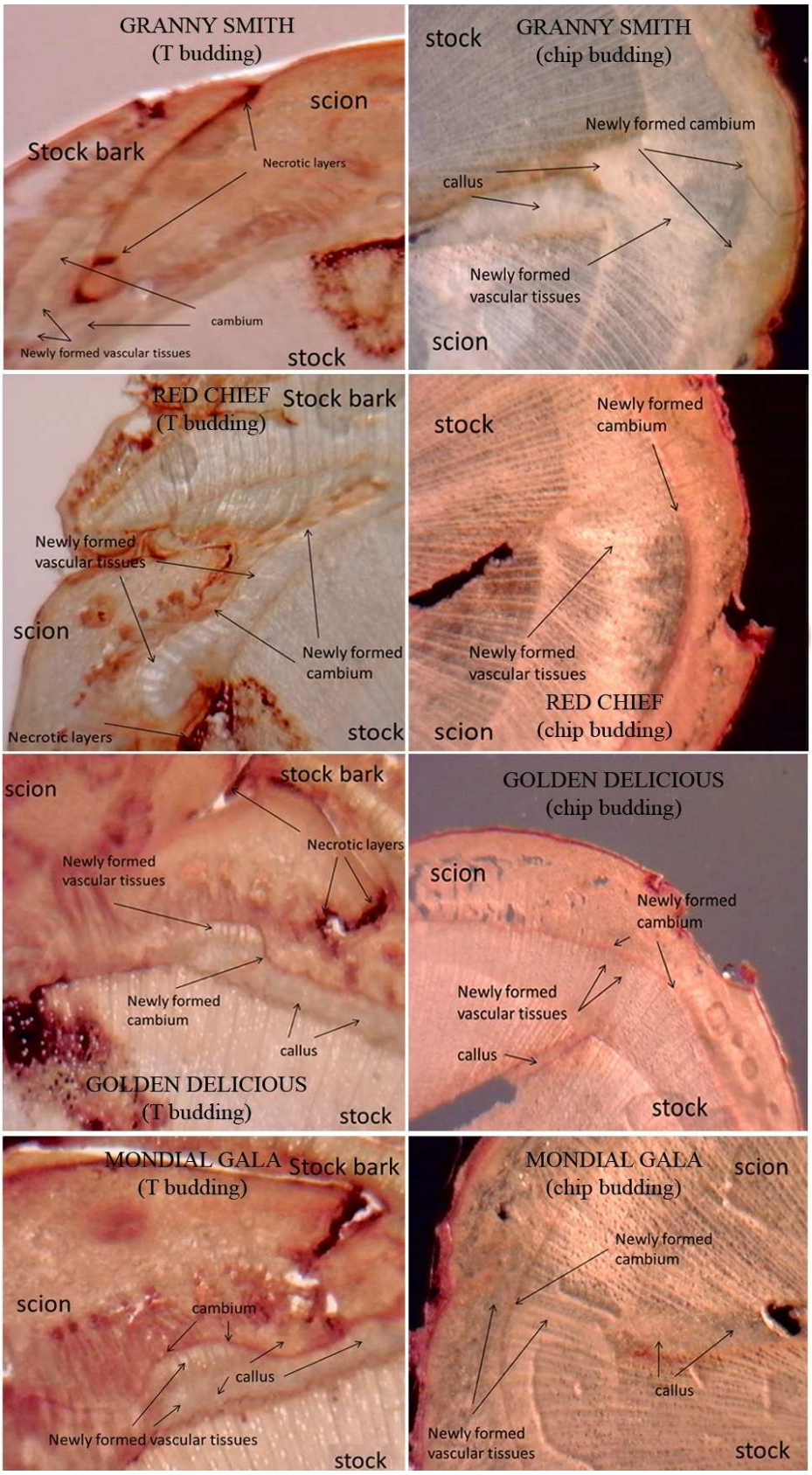

Fig. 4. *Horizontal sections from 90 days old samples of apple grafted on seed rootstock*

Fig. 5. *Photographs shoving the graft union development with different budding and apple cultivars on seed rootstock (90 days after grafting)*

The present results indicated that chip budding was more suitable method than T budding for apple sapling. Similarly, Kviklis (1986) reported that chip budding produced higher grafting success in apple and some other fruits in a five years field study. Küden (1988) reported that sprouting rate was 81.67% and 70.0% for Anna and Stark Earliest cultivars grafted with growing season grafting onto MM106 apple rootstock under subtropics climatic conditions. Besides, chip budding was found to be more effective than T budding in terms of bud take and sprouting in different fruit species. Similar results were also reported by Howard et al. (1974), Alibert and Masseron (1976), Stoyan (1984), Czarneck (1990). Skene et al. (1983) reported that chip budding was found to be more effective than T budding in terms of bud take and sprouting in apple and pear. They observed in crosscut from graft union that the blanking between epidermis of rootstock and scion delayed the integration.

Mean temperature varied with 13-28 °C between days after grafting in trial place (Bolu). This temperature range did not affect the grafting combination. Besides, as in accordance with Dolgun et al. (2008) results, the high altitude of Bolu did not affect the grafting combination. Also, Hartman et al. (2002) reported that temperature affected grafting integration significantly. Callus induction increased linearly between 4 °C and 32 °C and stopped exactly under 0°C in apple grafting. Yılmaz (1992) reported that 26-28°C temperatures were optimum for callus induction. Besides , humidity and oxygen are also important for parenchyma cells forming thin layed and sensitive callus tissue.

In this research, a complete grafting success was obtained from all combinations grafted with chip budding. Effectiveness of the method could be attributed that it caused the less necrotic tissue induction and cambial continuity and vascular connection were provided in short time due to a little gap between the rootstock – scion.

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