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Improved Success of Persian Walnut Grafting Under Environmentally Controlled Conditions

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ABSTRACT. Different budding methods (patch, shield and chip) were compared under controlled and field conditions using different native varieties of Persian walnut (*Juglans rejia* L.) during June 2005, in Nieriz, Fars province, Iran. Controlling temperature and humidity in a greenhouse improved grafting success considerably in all grafting methods compared with results under field conditions. The highest success rate was obtained with patch (91.0%) followed by shield (31.1%) and chip (19.1%) budding under greenhouse conditions versus 25.0, 15.0 and 10.0% under field conditions, respectively. Similar trends were also observed for callus formation and scion growth. Patch budding showed the highest callus formation and scion growth followed by shield and chip

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budding under both conditions. The genotype used did not affect grafting take using shield and patch budding excluding the N3 selection which had the highest graft success using the shield budding under greenhouse conditions. The best results were obtained by patch budding, under greenhouse conditions in all of the tested genotypes. doi:10.1300/J492v06n04_02

KEYWORDS. Walnut, grafting, variety, patch, shield, chip budding, greenhouse

INTRODUCTION

Persian walnut is one of the main nut crops in Central Asia, and is especially important in Iran (Vahdati, 2000). Iran was ranked as the third largest walnut producer in 2005 by producing 150,000 tons which is about 11% of the world walnut production (F.A.O., 2005; Vahdati, 2000). This reflects a trend of increasing walnut production in recent years (Vahdati, 2000). The Iran plateau has been regarded as the site of origin of the *Juglans regia* species in some literature and thus, this species is named "Persian walnut" (Leslie and McGranahan, 1998). Large populations of *Juglans regia* exist in this region, and most of them are seedlings and notably variable in production and nut quality (Germain, 1993; Ozkan et al., 2001; Reil et al., 1998; Stanisavljevic and Mitrovic, 1997). Selections of promising walnut cultivars in these populations along with the market demand for better quality products have increased interest in vegetative propagation of this species (Ferhatoglu, 1997; Vahdati, 2003).

Walnut trees are more difficult to graft than most fruit trees (Dhuria et al., 1977; Kuden and Kaska, 1997; Ozkan and Gumus, 2001) and poor graft take has always been considered a drawback in mass propagation of superior walnut selections (Ozkan et al., 2001; Roghani, 1977; Vahdati, 2003).

Environmental conditions during and after grafting, have a major impact on callus formation in walnut (Avanzato and Atefi, 1997; Rongting and Pinghai, 1993; Sitton, 1931). Optimally, the temperature should be maintained at about 27°C after grafting for better callus formation and graft success in walnut (Avanzato and Atefi, 1997; Germain, 1998; Reil

et al., 1998; Sen, 1986; Sitton, 1931). Accordingly, outdoor grafting is always restricted by the time of year when such favorable temperatures can be expected (Karadeniz, 2005; Kuden and Kaska, 1997; Hartmann et al., 2001). Different techniques of grafting have been examined by several researchers to improve the temperature and humidity effects by using controlled environmental conditions (Achim and Botu, 2001; Atefi, 1997; Avanzato, 2001; Avanzato and Atefi, 1997; Avanzato and Tamponi, 1987; Ferhatoglou, 1997; Lagerstedt, 1981; Lantos, 1990), however, most of these methods were inefficient, expensive, and not applicable on a large scale. Thus, existing procedures need improvement to allow efficient walnut propagation in nontraditional months of a year.

The objective of this study was to compare the efficiency of walnut budding under greenhouse versus field conditions for producing budded walnut plants in different months of a year and in different areas than those currently in use and to characterize the performance of different techniques of budding under controlled conditions in different genotypes.

MATERIALS AND METHODS

To compare the effects of different grafting methods and genotypes under greenhouse and outdoor conditions, two experiments were conducted in Neiriz, Fars province, Iran. In the first experiment, three types of budding, including patch, shield and chip were investigated under greenhouse versus field conditions using a selection named N1 during June 2004. In the second experiment, the performance of patch and shield budding were compared in different selections under greenhouse conditions. Scion buds were taken from five local selections (named N1, N2, N3, N4 and N5) which have been chosen from among 1000 trees in Neiriz, Fars province, Iran. All of the selections were lateral bearing and their kernel percentage was between 48 and 54%.

The top of the rootstocks was cut back at three stages. At first, the rootstock was partially cut back to about 10 cm above the bud, leaving about 5-6 leaves above the scion bud. Fifteen days after budding, the rootstock was cut back to two leaves above the graft union. The last cut back was done after bud burst leaving 8-10 cm above the bud scion to help prevent the new shoots from dieing back.

Since temperature and humidity have crucial effects on the healing process, they were recorded during the experiment using a maximum-minimum thermometer and a hygrometer, respectively (Figures 1 and 2).

FIGURE 1. Trend of daily temperatures 3 weeks after budding under field and greenhouse conditions.

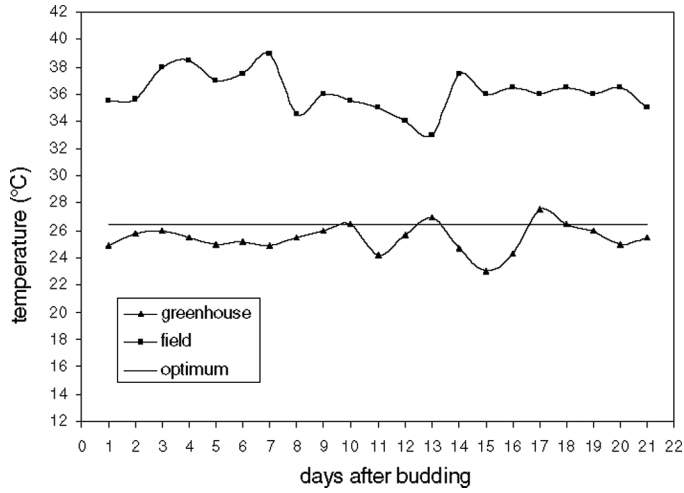
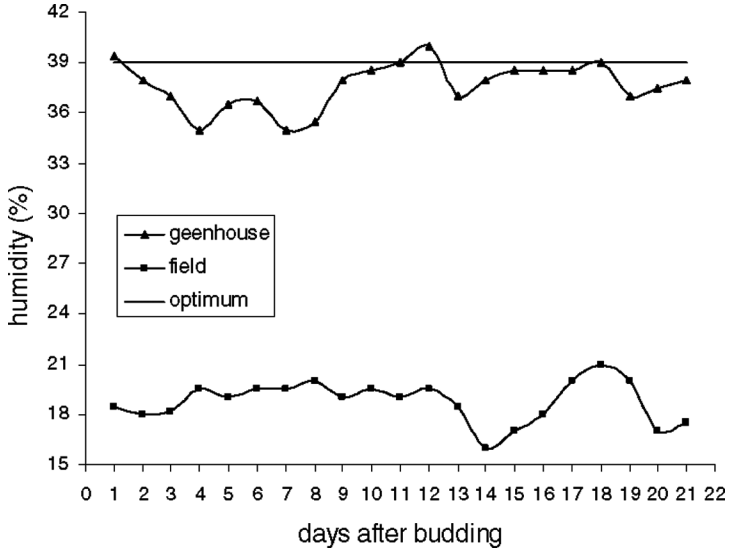


FIGURE 2. Trend of daily humidity 3 weeks after budding in field and greenhouse condition.



The mean temperatures in the greenhouse during the day and night were 25°C and 21°C and the mean relative humidity were 39 and 70%, respectively. These conditions were maintained in the greenhouse for 3 weeks after budding in. Humidity was controlled using watering the plants and surfaces during the day. The greenhouse roof surface was painted using the clay soil to reduce light received by the budded plants.

Field (outdoor) mean temperature was 32°C during the day and 17°C at night and relative humidity was 19 and 30% during the day and night of callusing process, respectively (Figures 1 and 2). To reduce direct light and temperature effects on the graft unions in the field, budding was done on the north-west side of the rootstocks. The top of the rootstocks were also bent on the junction portion to reduce light penetration. Light intensity ranged between 3,000 and 5,000 lux during the day in the greenhouse.

In the first experiment, data were combined and analyzed on the basis of a completely randomized design; and in next experiment, data were compared as a factorial experiment on the basis of completely randomized design. Statistical analyses were conducted using the SAS (SAS Institute, Cary, NC), and means were compared by least significant difference (LSD) at 0.05.

RESULTS AND DISCUSSION

Effect of Grafting Methods and Conditions

Interactions of graft type × condition were significant, excluding for rootstock diameter. Thus, graft type × condition interaction effects were compared using LSD method (Table 1). Rootstocks diameter did not show any significant difference, indicating the homogeneity of rootstocks in this experiment.

Percentages of "bud-take" were affected by different methods of budding under both conditions. The success rate of patch, shield and chip budding were 91, 31 and 19% in greenhouse versus 25, 15 and 10% under field conditions, respectively (Table 1). Under both greenhouse and field conditions, bud-take was highest using patch budding as compared with the shield and chip methods. Unal (1992) and Kaska et al. (1993) also obtained the highest success using patch budding. Lower bud-take of shield and chip budding could be related to less contact of the scion cambium with the rootstock bark cambium (Hartmann et al., 2001). We obtained a 19% bud-take success under greenhouse conditions

TABLE 1. Effect of different budding methods on callus formation, scion growth and grafting success percentage of N1 walnut selection under greenhouse (indoor) and field (outdoor) conditions.

Graft type	No. grafted plants	No. successful grafts	Callus formation[x] (0-3)	Rootstock diameter (cm)	Scion growth (cm)	Successful grafting (%)
Indoor						
Patch	178	162	2.87a[y]	1.13a	8.18a	91.0a
Shield	161	50	2.00c	1.62a	2.77b	31.1b
Chip	157	30	1.60e	1.20a	2.50b	19.1d
Outdoor						
Patch	400	100	2.83b	1.32a	3.45b	25.0c
Shield	400	60	1.80d	1.38a	3.10b	15.0d
Chip	100	10	1.00f	1.29a	1.80b	10.0e

[x]Callus formation rating from 1 (little callus) progressively to 3 (very good callus).
[y]Means with the same letter in each column are not significantly different.
Note: Mean separation within column by LSD at $P = 0.05$.

with chip budding which is in agreement with Gautman (1990). Patch budding success under field conditions was 25%, whereas Kuden and Kaska (1997), Gautman (1990), Nasiri (1994), Atefi and Mostafavi (1983) and Karadeniz (2005) reported a 50, 35, 55, 50 and 29-64% success rate, respectively. Variation in the success rate could be due to various environmental conditions in different areas (Figures 1 and 2).

Callus formation was higher under greenhouse conditions compared with outdoors using all budding methods. Patch budding produced the best callus formation and scion growth followed by shield and chip budding under both conditions (Table 1). Temperature and humidity have a pronounced effect on the production of callus tissue, which is essential for good graft union formation (Hartmann et al., 2001; Karadeniz, 2005; Vahdati and Zareie, 2006). Callus formation generally takes place between 4°C and 32°C; although maximum callusing in walnut has been reported at 26.5°C (Sitton, 1931) (Figure 1). Unal (1992) and Li et al. (1984) reported the highest walnut graft success at 27.8°C. In addition, since the parenchyma cells comprising the callus tissue are thin-walled and tender, they have no protection against desiccation, which is caused by low relative humidity and direct sunlight, and get readily injured (Hartmann et al., 2001). The results indicate that controlling temperature and humidity to around the optimum for callusing in greenhouse (Figures 1 and 2) improves the budding success. This confirms the results

of our previous work (Dhuria et al., 1977; Sharma and Dhuria, 1981; Vahdati and Zareie, 2006).

There is also evidence that high intensity light prevents callus formation (Hartmann et al., 2001). Under greenhouse conditions, painting the roof surface using the clay soil reduced light irradiance to 3,000-5,000 lux during the days and helped to maintain suitable temperature and humidity. Whereas high intensity sunlight, high daytime temperatures, and low humidity during late spring and early summer caused poor callusing in the field experiment. This is in agreement with Kuden and Kaska (1997) and Hartmann et al. (2001) who suggest that walnut budding be undertaken in late summer under field conditions found in subtropical regions to reduce adverse conditions.

Effect of Genotype

In the second experiment, graft type × genotype interaction effects were significant for callus formation and rootstock diameter. Therefore, the interactions of graft type and genotype were compared using the LSD method (Table 2). Selections did not show significant differences in graft success using patch and shield budding, excluding the N3 selection

TABLE 2. Influence of walnut selections on callus formation, scion growth and grafting success percentage using patch and shield budding under greenhouse conditions.

Variety	No. grafted plants	No. successful grafts	Callus formation[x] (0-3)	Rootstock diameter (cm)	Scion growth (cm)	Successful grafting (%)
Patch						
N1	178	162	2.87b[y]	1.13a	8.18b	91.0a
N2	153	131	3.00a	1.31a	9.53a	86.3a
N3	180	162	2.93b	1.13a	8.46ab	90.0a
N4	141	121	2.92b	1.15a	8.47ab	85.1a
N5	136	128	2.98a	1.37a	9.63a	88.2a
Shield						
N1	100	50	2.00d	1.62a	2.77c	30.0c
N2	75	30	1.80e	1.18a	3.37c	40.0c
N3	55	30	2.63c	0.87a	2.60c	54.5b
N4	94	30	1.90de	0.98a	3.70c	31.9c
N5	161	30	1.90de	0.95a	2.59c	31.1c

[x]Callus formation rating from 1 (little callus) progressively to 3 (very good callus).
[y]Means with the same letter in each column are not significantly different.
Note: Mean separation within column by LSD at $P = 0.05$.

which had the highest graft success using the shield budding. Grafting success of N1 was the highest (91%) and N4 was the lowest (85%) using patch budding in comparison with N3 (54.5%) and N1 (30%) using shield budding under greenhouse conditions (Table 2). Rootstock diameter at 5 cm above the crown was not significantly different in the selections, which indicates homogeneity of rootstock in this experiment. Scion growth after 45 days was the highest using patch budding compared with shield budding in all studied selections, which was related to earlier scion bud sprouting in patch budded plants. Scion growth of the selections was not significantly different for shield budding. However, the N1 selection had significantly lower scion growth compared to the N2 and N5 selections. According to the results of other researchers, bud-take percentage usually varies in different walnut selections (Kuden and Kaska, 1997; Stanisavljevic and Mitrovic, 1997), which is consistent with the obtained results in our experiment.

CONCLUSIONS

Favorable temperature and humidity are essential for callus development in walnut grafting; however, these factors are not usually favorable outdoors during many months of the year. Therefore, controlling temperature and humidity could have a major impact on walnut grafting success. Under greenhouse conditions we could control diurnal temperatures and relative humidity to meet the requirements for optimum callusing of the grafts. Our results also suggest that patch budding produces better callusing, scion growth and bud-take as compared with shield and chip budding. Therefore, we recommend producing grafted walnut plants using patch budding under greenhouse conditions for avoiding adverse outdoor conditions. This technique is also economically promising and allows for expansion into new areas and for propagation during nontraditional months.

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