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Introducing a Simple and Efficient Procedure for Topworking Persian Walnut Trees

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Abstract

To find the most suitable procedure for improving the quality and productivity of Persian walnut trees (*Juglans regia* L.) through topworking, different grafting methods were compared from 2001 to 2006. The effect of cultivar (scion) on grafting success was also assessed in a separate trial with the most successful method of grafting in 2004. Modified bark grafting of scaffolds performed in mid-April resulted in the highest graft take (100%) and survival (> 85%). Grafting success differed (0 to 100%) depending on the cultivar used as the scion. Other grafting (whip and cleft) or budding (chip, patch and I-shaped) methods on scaffolds produced poor results and/or no survival. The number of nuts, color and weight of kernels was superior in the topworked trees compared to the non-topworked ones ($P \leq 0.01$). The modified bark grafting method represents a simple and effective technique to convert inferior walnut trees to desirable cultivars. This technique included delaying grafting date for two weeks after rootstocks were cut back and temporarily covering the graft union with moist sawdust. After about three weeks, the sawdust cover was removed and the graft union was covered with grafting wax.

Grafting of Persian walnut (*Juglans regia* L.) is difficult compared to other fruit trees, and requires more precision in terms of grafting method, date, and selecting/handling of scions and stocks (1, 6, 7, 8, 9, 10,12). For this reason, walnut orchards have been traditionally established with seedling trees in many countries and as a result a large number of trees produce commercially unacceptable nuts (1, 4, 11). In view of the serious problems with walnut grafting and high cost of orchard replanting, as well as a high rate of mortality during transplanting of grafted or budded trees (8, 9, 12, 13), topworking seems to be the best way to improve nut yield and quality in previously established walnut orchards. Moreover, market and orchard management demands for specific cultivars bring a global need to find an efficient method of topworking (6).

Topworking is a type of grafting or budding that is completed at the scaffolds of the tree canopy or directly on the upper trunk in order to replace inferior cultivars with commercially acceptable ones (6, 8, 12). This method is traditional for several fruit crops (2, 5, 8, 13, 14). Various procedures of topworking have been suggested for walnut, including bark and cleft grafting on scaffolds or trunks during early spring (6, 7, 8, 9, 12), patch and chip budding, as well as whip grafting on the shoots arising from heavily cut back scaffolds or trunks (5, 6). To our knowledge, limited research has been reported on the long-term effectiveness of topworking under field conditions, and uncertainty remains regarding the best procedures for topworking, graft survival, yield and quality of topworked trees. The main objective of this study was to determine the most effective and simple method for topworking inferior mature walnut trees. In addition, the effect of cultivar on grafting success, and the impact of topworking on later nut yield and quality were assessed.

Materials and Methods

General description and cultural practices. The experiments were carried out in 5- to 20- year old Persian walnut seedling orchards (44°70' E, 37 ° 60' N, altitude 1325 m) from 2001 to 2006. During grafting time

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(early spring), average temperature and relative humidity were about 11.5°C and 52.0%, respectively. Soil in all orchards was sandy loam with pH of approximately 7.9. The experimental design in all experiments (except for the 2004 experiment) was randomized complete block with three replications and five trees per plot.

In all of the experiments, grafting was done as described by Hartmann et al. (6) and by the same grafter. For modified bark grafting, the grafting date was delayed for two weeks after rootstock heading-back and graft unions were covered with moist sawdust for about three weeks prior to waxing the graft. Sawdust (mainly of spruce and fir) was washed gently several times in a wooden container and finally rinsed with fungicide (1.5% (v/v) benomyl) and drained for approx. 48 h. A small amount of sawdust, on one side of a plastic bag, was used to cover the graft union and gently wrapped and fastened around the union. After about three weeks, the sawdust cover was removed and the graft union was covered with grafting wax. Based on our previous observations, covering the graft union with moist sawdust improves callus formation at the graft union (9).

Local cultivars named 'OR126' and 'Kahriz1' were used as sources of scion wood in all of the trials, except for the 2004 trial, which used 12 different local cultivars. Mother stock trees were severely pruned to stimulate suitable one-year-old shoots (scions) for all of the experiments except for the 2004 trial. Scions (dormant one-year-old shoots) were cut to 15 to 20 cm in length with at least two buds. Scions were then wrapped in damp paper, placed in a plastic bag and stored at 2 ± 2°C until they were used for bark grafting. For the rest of grafting/budding methods including cleft, whip, chip, patch, and I shaped , scions were taken from the mother trees just before grafting.

Data for percentage of graft take were collected 21 days after grafting, and survival rate was evaluated one year after grafting. The assessment of graft take 21 days after grafting is based on the percentage of scions which retained green bark color and plump, non-desiccated buds (6).To normalize the data, they were transformed by $\sqrt{x+0.5}$ (x is a number) before statistical analysis and means were separated using Duncan's multiple range test. Untransformed means are presented in the tables. The number of nuts per branch and average nut weight were recorded four years after topworking during harvest time. The color of kernels was rated using a scale of 1 to 6 (1= very light to 6= very dark) on 10 topworked and non-topworked branches and analyzed by the paired *t* test. SPSS software (SPSS, 2002) was used to analyze the data.

Experiments in 2001. In this preliminary experiment, the suitability of nine different topworking or topbudding methods was tested (Table 1). The tree part that was grafted and the time of grafting/budding differed for different methods. Patch and I- shaped budding were done after bark slipping (16-20 April) but chip and whip grafting were completed before bark slipping (25 and 26 March) using shoots 1.5 to 2 cm in diameter. Cleft and bark grafting methods were done either on main scaffolds (3-5 scaffolds) 3 to 10 cm in diameter, or trunks 5 to 15 cm in diameter before and after bark slipping, respectively. For trunk grafting, the entire trunk of the tree was cut off. Grafting was started in mid-March with cleft and whip grafting before bark was slipping, continued with bark grafting, and ended with I- shape and patch budding of new shoots arising from cut-back limbs in mid-April.

Experiments in 2002 to 2004. The two most promising topworking methods identified in the preliminary experiment (cleft grafting and modified bark grafting) were further evaluated for two successive years in 2002 and 2003 (Table 2). In a separate experiment in 2004, 10 to 15 scions from each of 12 local cultivars selected for higher yield and quality (Table 3) were topworked on the scaffolds of 10-year-old trees using the modified bark grafting method in mid-April, 2004, to compare the grafting success with different scion cultivars.

Results and Discussion

2001 experiment. The percent of graft take, three weeks after grafting, for all of the grafting methods used in the preliminary trial was very high (73 to 100) indicating a healthy start to growth (Table 1). However, many scions failed to continue growth and did not survive one year after grafting. This failure may be a result of high root pressure (sap bleeding) of walnut trees in early spring (6, 7, 8, 9, 12) or winter injury due to weak or too-fast growth of scions. The best survival one year after grafting was obtained with the modified bark grafting method (96%), followed by cleft grafting (37%). Other grafting (whip and bark) or budding (chip, patch and I-shaped) methods resulted in little or no scion survival after one year. Normal bark grafting was ineffective even if grafting date was delayed for two weeks after heading back the rootstock and immediate waxing of the graft union (7, 8, 9, 12), and only resulted in 20% survival when done on scaffolds (Table 1). In the case of successful grafts, the rate of scion growth seemed to have a dramatic effect on graft survival. Survival of grafts was higher (>93%) for scions grafted on scaffolds than trunks. The latter appeared to be more vulnerable to winter injury due to a very rapid growth rate (succulent growth) and most of them (>80 %) failed to survive the winter (Table 1). Growth of scions grafted onto the trunk was so rapid that they reached about 2.5 m in length by the end of growing season (data were not collected). Modified bark grafting of scaffolds resulted in a moderate rate of scion growth and higher winter hardiness and survival.

2002 and 2003 experiments. Further comparison of the cleft and modified bark grafting methods for two years revealed that only modified bark grafting resulted in good graft survival of about 93% and 76% in 2002 and 2003 experiments, respectively (Table 2). Therefore, considering the 96% success rate in the first experiment, modified bark grafting (Figures 1 and 2) was the most successful method over three experiments. We attribute this success to effective control of xylem bleeding and as a result providing better aeration and moisture content around the graft union. All these advantages were achieved mainly by temporarily covering the graft union with moist sawdust before waxing. Grafting delay for about two weeks alone, as well as complete waxing of the graft union, was not effective for improving grafting success. Waxing seems to inhibit

Table 1. Mean percentages of graft take and survival on walnut (*J. regia*) with nine topworking methods[z].

Topworking method	Tree part	Grafting date	Graft take[y] (%)	Survival[x] (%)	Scion growth rate
Cleft	Scaffolds	March 17	80.0b	36.7 b	Sustained to moderate
Chip	Shoots	March 25	73.3 b	0.0 d	-
Whip	Shoots	March 26	80.0 b	0.0 d	-
Normal bark	Trunks	April 13	80.0 b	0.0 d	-
Modified bark	Trunks	April 14	96.3 a	20.0 c	Very vigorous
Normal bark	Scaffolds	April 13	93.6 a	20.0 c	Moderate to vigorous
Modified bark	Scaffolds	April 14	100.0 a	96.3 a	Moderate to vigorous
I- shaped	Shoots	April16	80.0 b	0.0 d	-
Patch budding	Shoots	April 20	80.0 b	0.0 d	-

[z] The experimental design was randomized complete block with three replications and five trees per each plot. Means are average of fifteen trees.

[y] Grafting take and survival are percentage of scions which remained green and survived 21 days and one year after grafting, respectively.

[x] Means followed by the same letter are similar as determined by Duncan's multiple range test ($p<0.05$).

Table 2. Mean percentage graft take and survival of two promising topworking procedures done on the scaffolds of 10-year old walnut (*J. regia*) trees in two successive years[z].

Grafting Method	Grafting date	2002 Graft take[y] (%)	2002 Survival[y] (%)	2003 Grafts take (%)	2003 Survival (%)
Cleft	March 15	53.3 b[x]	20.0 c	46.6 b	0.0 b
Cleft	March 28	40.0 b	0.0 d	43.3 b	0.0 b
Modified Bark	April 1	93.3 a	33.3 b	50.0 b	0.0 b
Modified Bark	April 15	100.0 a	93.3 a	100.0 a	76.6 a

[z] The experimental design was randomized complete block with three replications and five trees per each plot. Means are average of fifteen trees (observations).
[y] Graft take and survival are percentage of scions which remained green and survived 21 days and one year after grafting, respectively.
[x] Means within a column followed by the same letter are not significantly different as determined by Duncan's multiple range test ($p < 0.01$).

callus formation as a result of frequent sap bleeding and air restriction under the wax cover (9).

On the other hand, doing the modified bark grafting about 15 days earlier (April 1) resulted again in very low grafting success (33%) only in 2002, and survival was zero in 2003 (Table 2). This poor result was due to a late spring freeze event, which occurred a few days after grafting and prevailed for about 7 days. A 17% reduction in graft survival in the case of modified bark grafting done in mid-April 2003 could also be attributed to a freeze event which occurred a few days after grafting. Therefore, to obtain maximum graft take and survival, grafting time must be sufficiently delayed to escape the risk of late spring frost and xylem bleeding. In general, based on multi-year observations, the best time for modified bark grafting in our climatic conditions was mid-April, a few days after bud break.

Cleft grafting, a commonly recommended technique for walnut (6, 8, 12), resulted in low grafting success (20 % only in 2002) (Table 2).

Figure 1. The modified bark grafting method as done on scaffolds of a 10-year-old walnut *(J. regia)* tree in mid-April, showing dormant scion secured under a bark flap with two nails (left) and covering with moist sawdust (right).

Table 3. Percentage of graft take and scion survival after modified bark grafting in mid-April 2004, using 12 local walnut (*J. regia*) scion cultivars.

Cultivar name	Graft take[z] (%)	Survival[z] (%)
Akbari	100.0[y]	87.5
Soofi	85.7	42.8
Milani	80.0	30.0
Alayari1	75.0	50.0
Alayari2	85.7	62.5
Zeinali	90.0	42.8
Islamdoost1	75.0	25.0
Islamdoost2	80.0	62.5
Kahriz1	100.0	70.0
Kahriz2	30.0	0.0
Kahriz3	80.0	50.0
Kahriz4	100.0	100.0
Mean of cultivars	81.7	51.9

[z] Grafting take and survival are percentage of scions which remained green and survived 21 days and one year after grafting.

[y] Each mean is the average of 10 to15 scions grafted onto scaffolds of two 10-year-old trees.

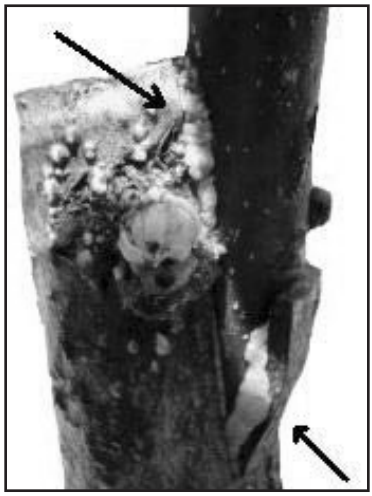

Figure 2. A close-up view of the graft union about 3 weeks after modified bark grafting, showing very good callusing. The arrows point to callus formation.

Moreover, cleft grafting was more difficult to complete than modified bark grafting and for these reasons we do not recommend it.

2004 experiment. Topworking of 12 different scion genotypes further affirmed the efficacy of the modified bark grafting method. Although graft survival varied from 0 to100% for different scion sources, on average grafting success was about 52% (Table 3). The variable grafting success could be attributed to the physiological status of mother stock (scion) trees (6, 7, 9, 12) rather than cultivar genetic make up as suggested by Stanisavljevic and Mitrovic (11). Modified bark grafting was also very effective (>90%) for grafting of two to three-year-old seedlings in the nursery (unpublished data).

General comments. The trees topworked in 2001 started to fruit 3 to 4 years later, but some cultivars ('Soofi' and 'Kahriz3') in the 2004 experiment fruited in the year after grafting, indicating early commencement of fruiting in topworked trees (8). However, fruiting should be avoided in the first and second year after topworking, because of negative effects on the tree's framework development ("runting") (3). Yield, kernel color and nut weight of topworked branches were significantly (p<

Table 4. Average number of fruits, nut weight and kernel color on trees topworked with 'Kahriz1' scions compared to non-topworked ones, measured at harvest time in October, 2006.

Yield and quality parameters	Topworked	Non- topworked	*t* test[x]
Number of fruits per branch	4.0[z]	1.8	3.71**
Nut weight (g)	5.8	4.1	8.48**
Nut color (scale from 1 to 6)[y]	1.8	3.5	2.76**

[z] Each mean is the average of 10 nuts in 10 paired 3-year-old branches from each of the topworked and non topworked trees.
[y] 1 = very light and 6=very dark
[x] **Indicates significant difference between topworked on non-topworked trees, as determined by paired *t* test at p< 0.01.

0.01) better than non-topworked ones in the fifth year after topworking (Table 4).

In conclusion, modified bark grafting in mid-April, with an average grafting success of about 85% in three successive years, could be considered as a simple and efficient method for replacing the tops of inferior walnut trees with promising genotypes/cultivars in order to improve yield and nut quality, and hence gain a higher market price.

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