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TOPWORKING OF MATURE WALNUT TREES AS AFFECTED BY  
DIFFERENT BLEEDING CONTROL METHODS AND SCION CULTIVARS

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KEY WORDS: *Juglans regia L.*, Bark grafting, Sawdust, Perlite, Cocopeat

**ABSTRACT**

Temporary covering of grafting point with moist sawdust has been reported effective on improving callus formation and grafting success in Persian walnut. In this study, we compared the effects of five kinds of grafting covers including sawdust, perlite, vermiculite, cocopeat and no covering (control) on callus quality, number of callused grafts, graft take, graft survival and scions growth. 'Pedro' and 'Chandler' were used as scion cultivars and modified bark grafting method was used to graft the main branches in the scaffolds of 10 to 15 years old walnut trees before mid April, 2008 and 2009. According to the results of first year experiment, among sawdust, perlite and no covering, the highest graft take (75.00%) and survival (66.04%) and the lowest graft take (10.00%) and survival (8.33%) was observed with sawdust cover and no covering, respectively. The results of second year experiment, among sawdust, coco peat and vermiculite showed the highest graft take (88.00%) and graft survival (84.67%) in sawdust cover treatment. Also the lowest graft take (38.33%) and survival (54.44%) was observed with vermiculite cover. The effect of scion cultivars was not statistically significant for all the studied characters except the scion growth in two years experiment. Our observations provide more support on the efficacy of moist sawdust for improvement of grafting success in Persian walnut.

**INTRODUCTION**

Traditionally walnut orchards have been established by seedling trees in most of walnut growing countries which limits orchard uniformity, profitability and causes production of large amount of commercially unacceptable nuts (Forde and McGranahan 1996; Stanisavljevic and Mitrovic 1997; Vahdati 2000; Achim and Botu 2001). In recent years higher market demands for high quality nuts and increased productivity of the superior walnut cultivars encouraged researchers to find satisfactory methods of vegetative production (Millikan 1971; Vahdati 2000). Vegetative propagation in walnut is very difficult and mostly limited to budding and grafting (Gautam 1990; Ferhatoglu 1997). However, walnut grafting is difficult as compared with the other fruit trees, and requires more attention in terms of grafting methods, date and selecting/handling of scions and stocks (Kuniyuki and Forde 1985; Rathore 1991; Rongting and Pinghai 1993; Vahdati 2000; Achim. and Botu 2001; Hartmann et al. 2001; Rezaee et al. 2008). Thus, propagation

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of superior walnut cultivars and selected genotypes has always been restricted by poor graft take of walnut trees (Ozkan and Gumus 2001; Vahdati 2003). In the other hand, higher cost of orchard replanting as well as higher rate of tree mortality during transplanting of grafted or budded trees were referred as major limiting factors in establishing of uniform orchards using grafted plants (Rathore 1991; Vahdati 2003; Rezaee et al. 2008).

Therefore, topworking of walnut trees is suggested as a good alternative method for walnut propagation and also to improve the yield and quality of previously established walnut orchards (Rathore 1991; Rezaee and Vahdati 2008). Unfortunately, limited research activities have been carried out around the world in relation to walnut topworking, in terms of suitable methods and conditions. In one study, topworking of 25 years old walnut trees resulted that chip budding was more promising compared to other methods with 58% grafting success (Gautam and Banyal 2005). A modified bark grafting method characterized mainly by temporary covering of grafting place with moist sawdust has been reported very effective to improve grafting success (>80%) both on mature and young walnut trees (Rezaee and Vahdati 2008; Rezaee et al. 2008). These reports claimed that sawdust cover absorbs xylem bleeding (sap) and as a result provides a moist and aerated condition in graft union suitable for better callus formation. Therefore, characterization of physical properties of sawdust and similar covers such as perlite, cocopeat and vermiculite would be very beneficial for developing a standard graft cover for commercial purposes. Thus, the main objectives of this experiment were to compare sawdust cover with another water absorbing materials (perlite, cocopeat and vermiculite) in terms of their effects on the callus quality, number of callused grafts, graft take, graft survival and scions growth as well as to understand their most important physical properties that affect on grafting success factors.

#### **MATERIAL AND METHODS**

The experiment was performed on 10 to 15 years old walnut seedling trees during April 2008 and 2009 in Department of Horticulture, College of Abouraihan University of Tehran (50.20 °E; 35.11 °N; altitude 940 m) with an ambient temperature and relative humidity of 17.6 °C and 27.17 %, respectively during callusing period.

In first year replication of experiment, three grafting covers include sawdust, perlite and plastic bag (control) were assayed on the grafting success factors. In second year replication, treatments were factorial combination of three grafting covers (sawdust, cocopeat and vermiculite). In both replications, scion cultivars of 'Pedro' and 'Chandler' were used. These experiments were done on completely randomized design with six treatments, in three replication and ten grafted scaffolds per plot.

Modified bark grafting method as described by Rezaee and Vahdati (2008) and Rezaee et al. (2008) was applied. All grafting operations were carried out by the same person. To cover grafting place, small amount of well drained sawdust and other mentioned materials was closely wrapped and fastened using plastic bag on the graft area. The mentioned covers (sawdust, perlite, vermiculite and cocopeat) washed gently and sterilized with fungicide (1.5% (v/v) benomyl) and were applied as above mentioned.

The scions woods were obtained in late January from walnut collection of Horticulture Department of Seed and Plant Improvement Institute (SPII) and were stored in damped cloths and plastic bags in a refrigerator (3 to 4°C) until they were used for topworking. Two weeks before grafting operation, rootstocks were headed back to minimize sap bleeding of trees (Kuniyuki and Forde, 1985; Rezaee et al., 2008).

After removing of covers, data were recorded for number of callused grafts, graft take and amount of callus formation (based on a visual scale of 1 to 4 in which 1= low, 2 =

medium, 3 = high and 4 = very high callusing). Subsequently, the percentage of graft survival (as a percentage of grafted branches where the graft took) and scion growth were measured for each treatment after 6 months. The data were analyzed using the SAS software (SAS Institute, Cary, N.C.)

## RESULT AND DISCUSSION

The results of both year experiments showed that grafting cover have a significant effect on the all studied characters. According to the results of first year experiment the highest callus quality (3.02 out of 4), callused grafts (86.66%), graft take (75.00%) and grafting survival (66.40 %) as well as the highest scion growth (83.12 cm) was obtained by sawdust followed by the perlite cover (Table 1). The lowest graft take (10.00%) was observed in no grafting cover. In the case of non-covered grafts, a higher amount of sap accumulated around the grafting union (Figure 1) and most of them (approx. 90%) were failed.

Also in second year experiment, the highest callus quality (3.7 from 4), callused grafts (95%), graft take (88.33 %) and grafting survival (84.37 %) as well as the highest scion growth (82.8 cm) was obtained in sawdust cover treatment followed by the cocopeat cover. In this trial, the lowest graft take (38.33%) and survival (54.44%) was observed with vermiculite cover (Table 1).

Accumulation of sap under grafting tape or wax could cause irreversible damage on actively dividing cells necessary for callus formation and as a result grafting failure in walnut (Rongting and Pinghai 1993; Rezaee et al. 2008; Rezaee and Vahdati 2008) and grapevine (Hartmann et al. 2001). There are also evidences that high intensity of light prevents callus formation (Hartman et al. 2001). Therefore, reduction of light may be another reason for increasing success in covered grafts.

In both trials, effects of cultivars on all studied parameters were non-significant, except on scions growth. This observation is in agreement with Rezaee and Vahdati, (2008) and is in contrary with the result of Rongting and Pinghai (1993), Stanisavljevic and Mitrovic (1997) who reported the graft-take varies in different walnut cultivars. It should be noted that grafting result could be affected by scion cultivars but this is mainly by scion quality which is a management related issue rather than genetic structure (Rezaee and Vahdati 2008). In both experiments of this study between used cultivars, 'Pedro' showed the highest scions growth compared to 'Chandler' (Figure 2).

In first year experiment, the interaction of grafting cover and cultivar was statistically non significant on studied characters except for graft take but in second year experiment interaction of grafting cover and cultivar was statistically non significant on all studied characters. In first year experiment the highest percentage of graft take (83.33%) was achieved by sawdust covered grafts in the 'Chandler' variety. Also the lowest percentage of graft take (6.66 %) was obtained by non-covered grafts in 'Chandler' variety.

In both experiments, percentage of graft take showed a positive correlation (first year  $R^2 = 0.73$ , second year  $R^2 = 0.89$ ) with callus quality as observed with different grafting cover and cultivars (Figure 3). This is in agreement with the result of Rongting and Pinghai (1993) and Rezaee et al., (2008) who reported that callus quality and amount of callus formation plays an important role in the grafting success.

According to the results, sawdust cover was better than perlite for absorbing of sap. Sawdust likely providing a suitable condition for callus formation and scions growth without any wood-rot around the graft area as reported by Rezaee et al. (2008) (Figure 4). Physical properties of used covers showed that the water holding porosity of sawdust is more than the perlite and vermiculite but less than cocopeat, which demonstrating that

sawdust and cocopeat provide a better condition by xylem sap absorbing and optimum moisture preparation. On the other hand, sawdust and cocopeat had lower aeration porosity compared to perlite and vermiculite but the experiment results indicated the sufficiently and suitability of this amount of aeration for callus formation (table 2).

### CONCLUSION

This is verified with our results that covering of grafting places specifically by moist sawdust increases grafting success in topworking of walnut. The obtained results of bleeding control method are promising and helpful for making special grafting tapes and/or prefabricated covers which provide suitable condition for grafting success artificially. Future researches could be focused on optimization and improvement of grafting condition by controlling of moisture, size and quantity of used sawdust also using of any other materials such as sponge for covering the graft point during the callus formation period.

### ACKNOWLEDGMENT

We thank Dr. Reza Amiri from Department of Agronomy and Crop Breeding, College of Abouraihan, University of Tehran for his assistant in analyzing the data. University of Tehran and Iran National Science Foundation (INSF) are also acknowledged for their financial supports.

### REFERENCES

1. Achim G, Botu I 2001. Results in walnut propagation using different methods. Acta Hort. 544: 503-509.
2. Ferhatoglou Y 1997. The studies on the effect of potting and omega grafting in relation to different time on graft taking percents of some standard walnut varieties. Acta Hort. 442: 303-308.
3. Forde H, McGranahan GH 1996. Walnuts. PP. 241-274. In: J. Janick and J.N. Moore (eds.) Fruit breeding. Vol. III: Nuts. John Wiley and Sons, New York.
4. Gautam DR, Banyal A 2005. Suitability of shoots portion and methods of summer budding/grafting in topworking of walnut. Acta Hort. 705: 319-323.
5. Hartmann HT, Kester DE, Davies FT, Geneve R 2001. Hartmann and Kester's plant propagation: principles and practices. 7 th edition. Prentice Hall, New Jersey. pp.880.
6. Kuniyuki AH, Forde HI 1985. Propagation. PP. 38-45. In: D.E. Ramos (ed.). Walnut orchard management. Division of Agriculture and Natural Resources, University of California, Davis, USA.
7. Millikan DF 1971. 'Propagation of *Juglans* species by fall grafting. Annual Report: Northern Nut Growers Association. 61: 41-44.
8. Ozkan Y, Gumus A 2001. Effects of different applications on grafting under controlled conditions of walnut. Acta Hort. 544: 515-520.
9. Rathore DC 1991. Walnuts. PP. 377-414. In: S.K. Mirta and D.C. Rathore (ed.). Temperate fruits. Calcutta, India.
10. Rezaee R, Vahdati K 2008. Introducing of a simple and effective procedure for topworking Persian walnut tree. Journal of the American Pomological Science 62: 21-26.
11. Rezaee R, Vahdati K, Grigoorian V, Valizadeh M 2008. Walnut grafting success and bleeding rate as affected by different grafting methods and seedling vigour. J. Hort. Sci. Biotech. 62(1): 21-26.
12. Rongting X, Pinghai D 1993. A study on the uniting process of walnut grafting and the factors affecting. Acta Hort.

13. Stanisavljevic M, Mitrovic M 1997. Effect of variety on successful grafting and development of nursery trees of walnut (*Juglans regia* L.). Acta Hort. 442: 281-284.
14. Vahdati K 2000. Walnut situations in Iran. Nucis Newsletter 9: 32-33.
15. Vahdati K 2003. Nursery management and grafting of walnut. Khaniran Publ., Tehran, Iran. PP. 99.

**Table 1.**

Callus quality, number of callused grafts, graft take, graft survival and scion growth in different sap bleeding control methods.

Year	Cover type	Callus quality <sup>A</sup>	Callused grafts (%)	Graft take (%)	Survival (%)	Shoot growth (cm)
1 <sup>st</sup>	Sawdust	3.02 a <sup>B</sup>	86.66 a	75.00 a	66.04 a	83.12 a
	Perlite	2.08 b	55.00 b	40.00 b	52.22 a	78.46 a
	Only plastic bag	0.94 c	21.66 c	10.00 c	8.33 b	13.32 b
2 <sup>nd</sup>	Sawdust	3.37 a	95 a	88.33 a	84.67 a	82.83 a
	Cocopeat	2.59 b	66.66 b	58.33 b	68.19 b	70.33 ab
	Vermiculite	1.76 c	48.33 b	38.33 b	54.44 b	59.66 b

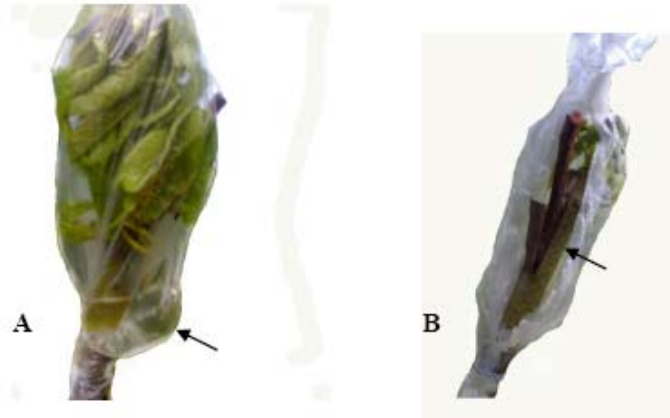
<sup>A</sup>Values are means of callus scoring ratings from 1 (low callus) to 4 (very good callus).

<sup>B</sup>Means with different letters in each column are significantly different at  $P \leq 0.05$ .

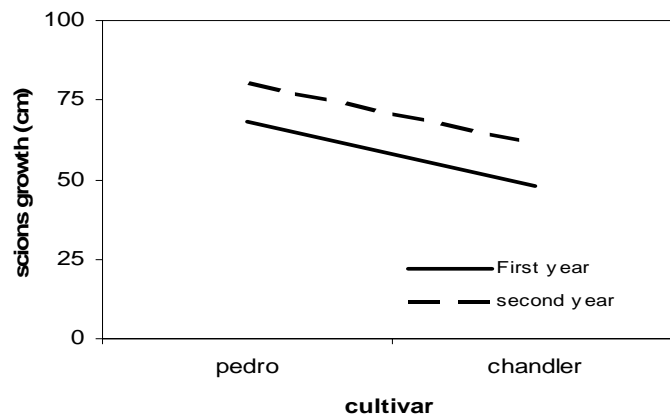
**Table 2.**

Effective physical properties and porosities in used covers (sawdust, perlite, cocopeat and vermiculite)

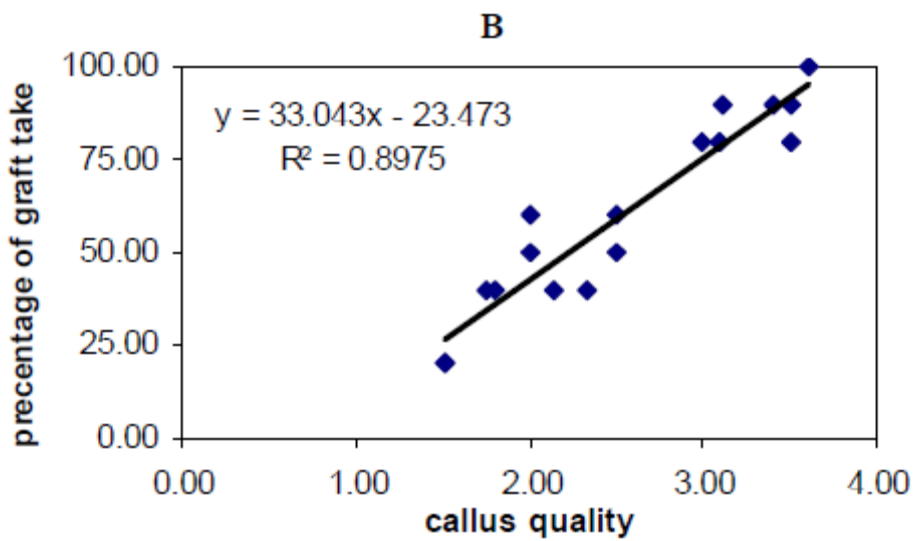
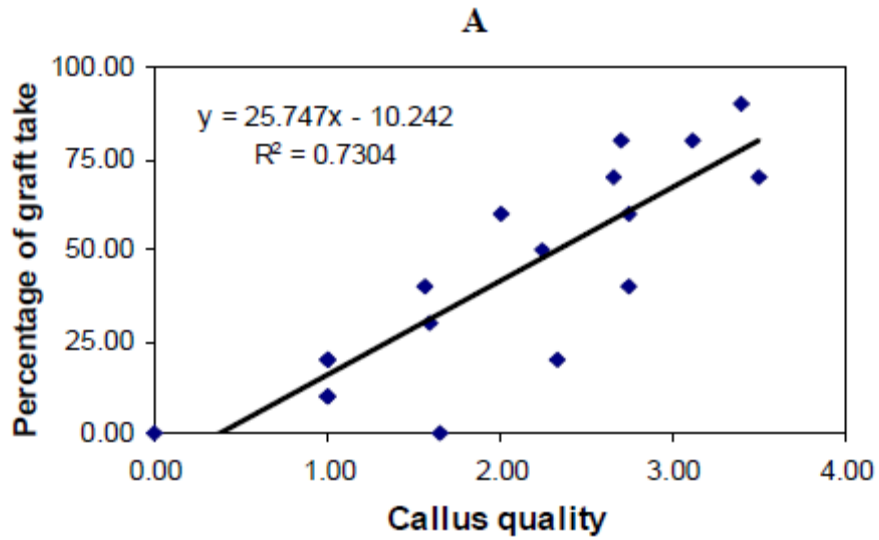
Type of covers	Total porosity (% by vol)	Water-holding porosity	Aeration porosity
Sawdust	56	49.72	6.28
Perlite	45	23.29	21.71
Cocopeat	78	76	2
vermiculite	50	31.8	18.2



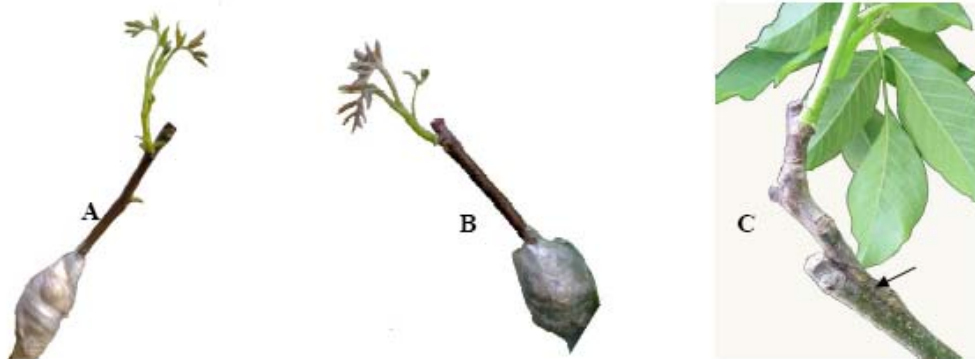
**Fig. 1.** A) High root pressure and accumulated sap under plastic bag. B) grafting failure in non-covered grafts after callus formation period.



**Fig. 2.** Effects of cultivar type ('Pedro' and 'Chandler') on scion growth in two years experiments



**Fig. 3.** Correlation between callus quality and percentage of graft take in different bleeding control methods and cultivars. A) First year replication B) Second year replication.



**Fig. 4.** A & B) Covered graft with sawdust C) scions growth and callus bridge formed.