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Effect of Budding Method, Rootstock Age and Cut below Budding Union on Budding Success in Persian Walnut

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Abstract

Budding is a good method of propagation in the Persian walnut (*Juglans regia* L.)., However, success of budding is low due to higher root pressure as well as the presence of phenolic compounds in the xylem sap. This study was conducted to compare various budding methods and cut below budding union on two and three-year-old Persian walnut under field conditions. The experiment was conducted based on a completely randomized block design with three replications. The first factor of experiment was budding methods at three levels (T-budding, invert T-budding and patch budding). The second factor was rootstock age at two levels (two-year and three-year old rootstocks).The third factor was to make cuts in the rootstock below budding union at two levels (cut below budding and no cut below budding). The results showed that budding method and rootstock age have significant effect on early percentage of budding success. The highest percentage of budding success (100%) was observed in three-year-old rootstock with T and inverted T-budding as well as in two-year-old rootstock with patch budding and the least success were gained in inverted T-budding on two-year-old rootstocks (69%). The highest callus formation was observed in patch budding on two-year-old, which is not differed by cut below budding treatment. The least callus formation was observed in T-budding without cut on two-year old rootstock (38%). Budding methods have a significant effect on secondary success (survival) at 1%. Patch budding method with 69% survival compared to other methods had most secondary success. Generally, in this study, patch budding had more callus formation, primary and secondary success compared to other budding methods.

Keywords: Budding, Callus formation, Grafting, Persian walnut.

Introduction

The Persian walnut (*Juglans regia* L.) is one of the most important commercial species in many parts of the world. Iran produces 453988 tons of walnut in 2013, which is ranked second in the world (FAO, 2013). Since walnut trees are largely genetically heterozygous, non-grafted seedling trees produce different quality (Gandev, 2007). Therefore, the most appropriate and common method to propagate walnut trees is asexual reproduction methods that creates uniform trees (Hartman *et al*, 2002). Grafting and budding are the most efficient methods for walnuts asexual reproduction. Almost all grafting methods can be used for walnut (Vahdati, 2003).

The survival rate in different grafting methods depends on grafting conditions and grafter skills. The most common method of Walnut budding and grafting are patch budding and side and side-tongue grafting, respectively. The grafting success and survival rate of grafted walnut seedlings are reported in the literature (Rezaee and Vahdati, 2008). Grafting success in

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walnuts is far less than other fruit trees (Weber and Mac Daniels, 1969). There are high concentrations of polyphenolic compounds and root pressure in walnuts trees, and this is the most important factors which reduces propagation success of this plant by grafting and budding. High concentrations of phenolic compounds lead to tissue browning after cut and prevent callus formation at grafting union (Pinghai and Rongting, 1993).

Therefore, with the hypothesis that cut below budding union cause's secretion of phenolic compounds and reduce the root pressure, it is possible that this action is effective in increasing grafting success. Other factors affecting budding success is budding methods, time of budding, plant genotype, environment temperature and humidity (Gandev and Arnaudov, 2011). Therefore, many efforts have been made to find a suitable method and rootstock for walnut budding. Type of rootstock is critical to the success of budding. For budding, rootstock must be strong and well-watered until skin is easily separated from wood. In this method, topping of rootstock has a good influence on growth of the scion (Vahdati, 2003).

The objective of this study was to determine the effects of budding method, age of rootstock and cut below budding and no cut below budding union on budding success and callus formation in Persian walnut.

Materials and Methods

The study was conducted in private nursery in Shahmirzad. The rootstocks were selected from seed genotypes of *J. regia* 'Chandler' that their diameter was 2.25 for two-year old rootstocks and 3.8 for three-year-old rootstocks. Scions were selected from large lateral buds (dormant) from Chandler.

To do the budding in all three methods (T-budding, invert T-budding and patch budding), terminal buds were removed in the rootstocks. In T-budding, a horizontal incision was made at the top of bud. Then, the cut was extended in both sides to meet together under the buds. This was similar to T-budding in that both methods of budding followed the same incisions on the rootstock and bud stick except that in inverted T the horizontal cut was made at the bottom of the vertical cut. Then, the incision was extended in the both sides to meet together above the buds and a cut on the rootstock was made as inverted T (⊥). The bud patch was prepared by two horizontal cuts about 2.5 cm apart (the same length as in the rootstock) in circular motion around the stem. Then, two cuts were connected by a vertical cut and the patch of bark was separated intact from the wood. On the rootstock, only 5-6 leaves were kept above the graft union for photosynthesis and to prevent excessive transpiration and to help budding success. The experiment was conducted based on a completely randomized block design with three replications. The first factor of the experiment was budding methods at three levels (T-budding, invert T-budding and patch budding). The second factor was rootstock age at two levels (two-year old and three-year-old rootstocks). The third factor was cut below budding union at two levels (cut and no cut below budding). The cuts were made 1 to 2 cm below the graft union. The measured parameters, including percentage of callus formation, indicate that this trait is only indicative of seedlings situation that callus is formed in graft union. This factor depends on the strength and growth conditions of rootstock before grafting. The early success of budding was measured by buds that grew in scion after 4 weeks from the time of budding. In this study, the percentage of trees that survived 8 weeks after budding was recorded as survival rate.

The data was analyzed by SAS software and means comparison was done by Duncan's multiple range tests. Tables and figures were drawn with Excel software (Fig. 1)

Fig.1. From right to left: T-budding without cut on two-year old rootstock, inverted T-budding with cut on three-year old rootstock and patch budding without cut on three-year old rootstock

Results

Early success

The results showed that the type of budding methods, cuts below budding union and rootstock age, had significantly different effects on callus formation, early success in budding and secondary success (survival of budded seedlings). According to the results shown in Table 1, the interaction of rootstock age × budding method and rootstock age × cut below the budding was significant at 5% level. Interaction of cut below the budding × budding method and rootstock age × budding method × cut below the grafting did not have a significant effect on the early success (Table 1). The mean comparison results showed that three- year old rootstock had 100% success in T-budding and Invert T-budding. Two-year old rootstock and three-year rootstock had 100% and 90% success in patch budding, respectively. The least success was observed in T-budding on two-year old rootstock at 69% (Table 2).

The results showed that cut or not cut at three-year old rootstock had no significant effect on budding success, while two-year old rootstock was significantly different in this treatment and not cut was more effective with 92% success (Table 3).

Table 1. Analysis of effects of different rootstock age, budding method and cut below the budding union on callus formation and budding success in walnut

S.O.V	df	MS		
		Early success	Callus formation	Secondary success
Block	2	0.083^{ns}	0.75^{ns}	0.19^{ns}
Budding method	2	1.75^{ns}	6.33^{*}	26.02^{**}
Rootstock age	1	10.03^{**}	0.44^{ns}	0.69^{ns}
Cut below budding	1	4.69^{*}	7.11^{*}	0.25^{ns}
Rootstock age × budding method	2	4.86^{*}	3.44^{ns}	4.52^{ns}
Budding method× cut below budding	2	2.16^{ns}	2.11^{ns}	1.75^{ns}
Rootstock age × cut below budding	1	4.69^{*}	7.11^{*}	8.02^{ns}
Rootstock age × budding method× cut below budding	2	2.19^{ns}	7.11^{*}	0.36^{ns}
Error	22	0.87	1.27	2.07
C.V	-	14.93	19.84	45.87

*, ** = Significant at 5 % and 1%, respectively, ns= Non-significant

Table 2. Interaction effect of rootstock age × budding method on early success.

Budding method / Rootstock age	T-budding	Inverted T-budding	Patch-budding
Two-year rootstock	76.14[b]	69[b]	100[a]
Three-year rootstock	100[a]	100[a]	90.43[ab]

In each column, means with the same letters are not significantly different

Table 3. Interaction effect of rootstock age× cut below the budding on early success

Rootstock age / Cut below budding	Two-years old rootstock	Three-years old rootstock
Cut	71.42[b]	96.85[a]
Not cut	92.14[a]	96.85[a]

In a column, means with the same letters are not significantly different

Callus formation

The results showed that the interaction of root-stock age × budding method× cut below the budding has a significant effect on callus formation at the level of 5% (Table 1). A comparison of means also showed that the highest percentage of callus formation was observed in the patch budding with and without cut on two-year old rootstocks and T-budding without cut on two-year old rootstock (100 %). The lowest callus formation was observed in T- budding without cut on two-year old rootstock (38%) (Table 4).

Table 4. Interaction effect of rootstock age × budding method× cut below the budding on callus formation

Rootstock age	Two-years old rootstock		Three-years old rootstock	
Cut below the budding / Budding method	Cut	Not cut	Cut	Not cut
T-budding	38[b]	100[a]	90.57[a]	76.28[ab]
Inverted T-budding	62[ab]	76.28[ab]	71.42[ab]	85.71[a]
Patch -budding	100[a]	100[a]	85.71[a]	85.71[a]

In a column, means with the same letters are not significantly different

Secondary success (survival)

According to analysis of variance table, only the budding method showed a significant difference at 1%, and the rest of factors had no significant effect on secondary success (survival) (Table 1). Results of mean comparison showed that patch budding method with 69% survival compared to other methods had most secondary success, but T-budding and inverted T-budding had not significant difference together (Table 5). In two and three-year's rootstocks, the survival rate did not show significant differences.

Table 5. Effect of budding method on secondary success

Budding method	Secondary success
T-budding	30.85[b]
Inverted T-budding	34.42[b]
Patch-budding	69[a]

In a column, means with the same letters are not significantly different

Discussion

The results obtained suggest that strong rootstocks are better for budding. Simultaneous activation of the rootstock and scion is a key factor affecting on budding success. Due to the activity of this layer, cell division is done and formed callus tissue. If the root is not active, the buds break, which results in evaporation of water and thus, the scion, dries quickly. Rootstock must be strong enough to easily peel the bark from wood (Vahdati, 2003). Two- year rootstocks are needed to develop a fibrous and strong root system. Therefore, budding success on two-year old rootstock is more than annual rootstock (Lantus *et al*, 1990). The results also proved that the use of three-year old rootstock compared to two-year old rootstock is the best for budding. The positive effect of use of highly growth and strong rootstock in increasing success also has been reported by other researchers (Rezaee *et al*, 2008). This effect is mainly due to the ability of rootstock to absorb water and nutrients and store higher carbohydrates in tissues (Jacobs *et al*, 2006).

Callus formation is an important factor for healing budding union and success in budding (Hartman *et al*, 2002). It resulted in a relationship between rootstock and scion and carried out complete vascular contact between scion and rootstock and growth of scion buds. (Hartman *et al*, 2002). In this research, patch budding had more callus formation and primary and secondary success compared to other budding methods. Our results correlate to Ebrahimi *et al*. (2006), who reported that under the greenhouse condition, the success of patch budding, T-budding and chip budding was 90%, 31% and 19% and in field condition, success in this methods was 25%, 15% and 10%, respectively. In their study, patch budding had higher survival, callus formation and growth of scion.

Conclusions

The results of this study showed that despite various reports about low achievement in walnut budding, using better budding methods and stronger rootstock can create higher success. Thus, we propose the use of the three-year old rootstocks and patch budding method for more success in walnut propagation. In this study, it was also found that cut below the budding union in two- year old rootstock and T-budding method had no positive impact However, in the three-year old rootstocks, cut below the budding union caused more callus formation and thus better budding success. Hence, cut below the budding can be used for budding of three-year old rootstock and strong rootstocks.

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