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OPTIMIZING ENVIRONMENTAL CONDITIONS AND IRRIGATION REGIMES CAN IMPROVE GRAFTING SUCCESS IN PERSIAN WALNUT

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ABSTRACT

The propagation of Persian walnut (*Juglans regia*) using grafting or budding is often difficult due to environmental factors. Temperature fluctuation and plant water status affect root pressure (xylem sap exudation "bleeding") during budding or grafting. An experiment was conducted to find the optimum irrigation regime for patch budding (in July) and tongue grafting (in March) of Persian walnut on *Juglans regia* rootstock under three environmental conditions (greenhouse, shade-house and outdoor). The grafted/budded plants were subjected to three irrigation regimes including 1-day, 3-day and 7-day for 4 weeks after grafting/budding. Callus formation and quality, and graft/bud-take were recorded after four weeks. The survival rate and growth of the scions were measured after three months. Based on the results, grafting success under greenhouse, shade-house and outdoor conditions were 66.7%, 42.6% and 17.6%, respectively. The highest graft/bud take (94.4%) was obtained under greenhouse conditions with patch budding. The lowest grafting success (0%) was observed under outdoor conditions with patch budding. Under the greenhouse conditions, irrigation of the grafted plants with 7-day interval resulted in the highest graft success and plant growth. Irrigation with 3-day intervals was the best regime under shade-house conditions, while daily irrigation regime was required to obtain the highest grafting success for the outdoor grown plants. In general, the present experiment revealed that modifying outdoor conditions by applying a shade-house equipped by a mist system in warmer area can improve bud-take and success especially in patch budding by 40.76 and 33.1 %, respectively.

Key words: shade-house, graft success, callus quality, irrigation regime, *Juglans regia* L.

INTRODUCTION

Persian walnut (*Juglans regia* L.) as one of the main nut crops is grown in large parts of the world [Vahdati 2000]. Since long time ago, propagation through seed was the only available method for walnut multiplication, though this practice resulted into plants with great variability. Therefore, grafting as the most widespread method has being used all around the world to propagate clonal plants [Sharma et al. 2003, Vahdati and Zareie 2006].

Environmental factors such as temperature and relative humidity (RH) directly affect on callus formation and grafting success in walnut [Sutyemez 2007, Dehghan et al. 2010, Soleimani et al. 2010, Sadeghi Majd et al. 2019]. Aeration in graft points is necessary

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for cell division and callus formation [Hartmann et al. 2001, Vahdati 2007]. The variations in graft success largely depend on different environmental conditions to which the plants are subjected before and after propagation [Chase 1947, Awasthi et al. 1984, Vahdati and Zareie 2006]. The best and most effective results under continental climate conditions were reported by bench grafting using the whip and tongue technique [Dehghan et al. 2009, Paunovic et al. 2011]. In some trees such as walnut, xylem sap exudation "bleeding" causes suffocation and death of cells in the graft site [Hartmann et al. 2001, Vahdati 2007]. Fluctuations in temperature and irrigation may intensify the harmful effects of high root pressure through bleeding and reduce grafting/budding success in walnut [Ramos 1985, Vahdati 2007]. Therefore, the present study was carried out to determine the optimum irrigation regimes for walnut grafting success under greenhouse, shade-house and outdoor conditions.

MATERIALS AND METHODS

The experiments were conducted in the experimental greenhouse, shade-house and field of Department of Horticulture, Aburaihan Campus, University of Tehran, Pakdasht, Tehran, Iran. Tongue grafting and patch budding methods were done in March and July 2013, respectively. The grafted seedlings were kept under three conditions: greenhouse, shade-house and outdoor. Three irrigation regimes including 1-day, 3-day and 7-day were applied for four weeks after grafting/budding.

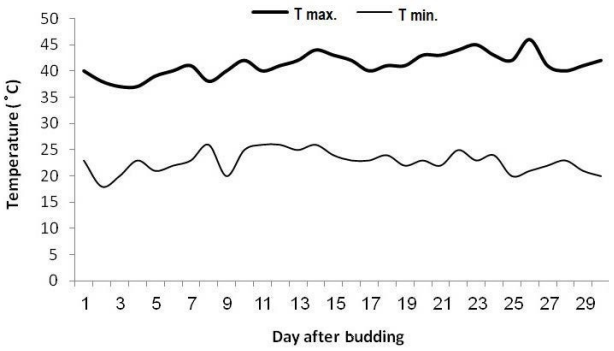

Fig. 1. Maximum and minimum temperatures during four weeks after patch budding of Persian walnut under outdoor conditions (T – temperature)

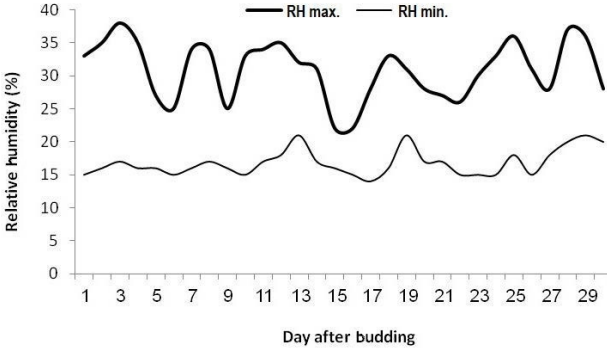

Fig. 2. Maximum and minimum relative humidity during four weeks after patch budding of Persian walnut under outdoor conditions (RH – relative humidity)

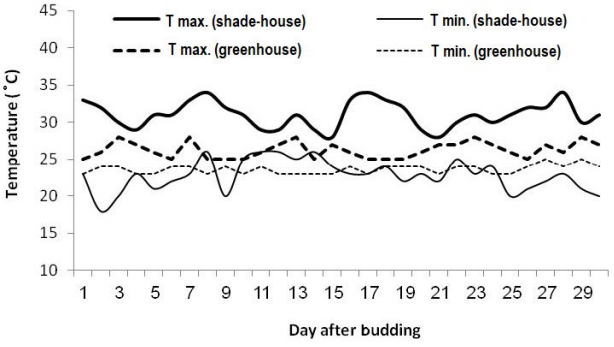

Fig. 3. The maximum and minimum temperature during four weeks after patch budding of Persian walnut under greenhouse and shade-house conditions

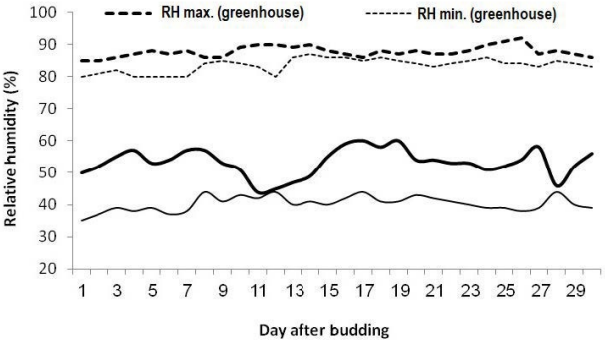

Fig. 4. The maximum and minimum relative humidity during four weeks after patch budding of Persian walnut under greenhouse and shade-house conditions

During budding, and for four weeks after budding, temperature (Fig. 1) and relative humidity (RH) (Fig. 2) of the outdoor conditions were recorded. At the same period, maximum and minimum temperatures (Fig. 3) and RH (Fig. 4) inside of the greenhouse (maintained at 25 ±2°C and 80–90%, respectively) and shade-house were recorded. Also, average of maximum and minimum monthly temperature and RH was recorded (Tab. 1).

Fifty percent of sunlight was kept out for the rootstocks under shade-house conditions. A mist system was used to spray water from above the grafted seedlings every half an hour for thirty seconds from 9 a.m. to 19 p.m. Soil texture was Silty loam with pH 7.3. In both conditions, shade-house and outdoor, rootstocks were planted in nursery (Fig. 5), but in greenhouse conditions seedlings were planted in 30 × 35 cm plastic pots, and two-year old walnut seedlings of a cluster bearing genotype were used as rootstock. In this study, 'Chandler' cultivar (as one of the best commercial walnut cultivars in the world) was used as a scion for both grafting and budding methods. Buds of walnut 'Chandler' were taken from basal and middle portions of one-year-old shoots three weeks and one day before tongue grafting and patch budding, respectively. The scions were treated with 0.3% fungicide Captan©, and the graft point was covered with cotton cloth adhesive tape after grafting/budding by four weeks.

Four weeks after grafting/budding, graft/bud-take and callus quality (based on a visual scale of 1 to 4 in which 1 = low, 2 = medium, 3 = high and 4 = very high callusing) were determined. Subsequently, graft/bud survival (%) and scion growth were measured for each treatment at the end of season and 3 months after grafting/budding, respectively. The experiment was performed as factorial on the basis of Randomized Complete Block Design (RCBD) with three replications and six grafts per replication.

Table 1. Mean maximum and minimum monthly temperature (°C) and RH (%) after budding

Conditions of budding	RH		Temperature	
	max.	min.	max.	min.
Greenhouse	87.7	83.5	26.2	23.6
Shade-house	53.3	40.4	34.0	22.8
Outdoor	30.9	16.8	41.2	22.8

Fig. 5. Planted seedlings in the second nursery for patch budding under shade-house (left) and outdoor (right) conditions

The data were analysed using the SAS software (1.6) and Duncan's multiple range test (DMRT) was used for mean comparisons ($P \leq 0.05$).

RESULTS

Grafting in greenhouse resulted in the highest, and grafting in the field resulted in the lowest callus quality, graft take, survival and scion growth (Tab. 2). In patch budding, the success was 43.2% (Tab. 3). The interaction between environmental conditions of grafting/budding and irrigation regimes was significant for all combinations and measurements (Tab. 4).

The interaction between environmental conditions and grafting/budding method showed highest-to-lowest survival rate obtained in patch budding under controlled and outdoor conditions with 77.8 and 5.55%, respectively (Tab. 5). Based on the results, the highest graft-take (94.5%) was observed in patch budding under the greenhouse conditions. Lower graft-take success was obtained under shade-house (27.5%) and the outdoor (0%) conditions.

The rate of scion height as an index of scion growth was significantly higher under shade-house conditions (117.3 cm) versus other treatments (Tab. 6). Also, weekly irrigation of the grafted/budded plants in the greenhouse was the best regime. Every three days of irrigation was the best regime under the shade-house condition while daily irrigation regime was the most efficient method under outdoor conditions.

DISCUSSION

The optimum temperature for callus formation in walnut under controlled conditions, which is necessary for graft healing, is about 25 ±2 °C [Avanzato and Atefi 1997, Achim and Botu 2001, Vahdati 2000]. In our study, temperature of the greenhouse was maintained between 25 ±2°C. Ebrahimi et al. [2007] reported improved budding success using controlled conditions. The highest success was obtained with patch (91%) followed by shield (31.1%) and chip budding (19.1%) under this conditions versus 25, 15 and 10% under field conditions. Graft success with patch budding in greenhouse was significantly more than the uncontrolled conditions [Ebrahimi et al. 2007]. We found the optimum grafting conditions in greenhouse verses shad-house and outdoor, with more success of grafting.

Table 2. Production success in Persian walnut grafting under different environmental conditions

Conditions of grafting/ budding	Callus quality [A]	Graft/bud-take (%)	Survival (%)	Scion height (cm)
Greenhouse	2.1[a]	66.7[a]	47.2[a]	89[a]
Shade-house	1.2[b]	42.6[b]	34.2[b]	80[a]
Outdoor	0.9[c]	17.6[c]	13.0[c]	54[b]

[A] Values are means of callus scoring from 1 (low callus) to 4 (very good callus)
Means with different letters in each column are significantly different according to DMRT at $P \leq 0.05$

Table 3. Production success in Persian walnut grafting using different methods of grafting/budding

Method of grafting/ budding	Callus quality [A]	Graft/bud-take (%)	Survival (%)	Scion height (cm)
Patch budding	1.3[b]	43.2[a]	30.8[a]	45.0[b]
Tongue grafting	1.4[a]	41.3[b]	32.1[a]	103.7[a]

[A]Values are means of callus scoring from 1 (low callus) to 4 (very good callus)
Means with different letters in each column are significantly different according to DMRT at $P \leq 0.05$

Table 4. Interaction of environental conditions and irrigation regimes on callus quality, graft/bud-take, survival and scion growth

Conditions of grafting/ budding × irrigation regimes	Callus quality [A]	Graft/bud-take (%)	Survival (%)	Scion height (cm)
G × I	1.70^{c}**	55.5^{bc}	33.3^{cd}	84.5^{ab}
G × II	2.10^{b}	63.9^{b}	50.0^{ab}	88.2^{ab}
G × III	2.60^{a}	80.5^{a}	58.3 ^{a}	94.7^{a}
S × I	1.30^{d}	41.7^{ed}	33.3^{cd}	78.8^{ab}
S × II	1.30^{d}	50.0^{cd}	41.7^{bc}	81.1^{ab}
S × III	1.10^{e}	36.1^{e}	27.8^{cd}	80.0^{ab}
O × I	0.95^{f}	22.2^{f}	19.4^{ed}	70.8^{bc}
O × II	0.95^{f}	16.2^{f}	11.1^{e}	16.2^{c}
O × III	0.73^{g}	13.9^{f}	8.30^{e}	35.0^{d}

G – greenhouse, S – shade-house, O – outdoor, I – irrigation regime daily, II – irrigation regime every 3 day, III – irrigation regime weekly
[A]Values are means of callus scoring from 1 (low callus) to 4 (very good callus)
Means with different letters in each column are significantly different according to DMRT at $P \leq 0.05$

Table 5. The interaction between condition of grafting and grafting method on callus quality, graft-take, survival and scion growth

Conditions of grafting/ budding × method	Callus quality [A]	Graft/bud-take (%)	Survival (%)	Scion height (cm)
G × P	2.3^{a}	77.8^{a}	51.85^{a}	81.0^{c}
G × T	1.9^{b}	55.5^{b}	42.6^{ab}	97.2^{b}
S × P	1.2^{d}	46.3^{c}	37.0^{bc}	44.0^{d}
S × T	1.3^{c}	39.0^{c}	31.5^{cd}	116^{a}
O × P	0.6^{e}	5.55^{e}	3.70^{e}	10.0^{e}
O × T	1.1^{d}	29.6^{d}	22.2^{d}	98.0^{b}

G – greenhouse, S – shade-house, O – outdoor, P – patch budding, T – tongue graftin
[A]Values are means of callus scoring from 1 (low callus) to 4 (very good callus)

In this study, the highest graft-take (94.4%) was obtained under greenhouse conditions with patch budding and weekly irrigation regime comparing to field condition (0%) with patch budding and weekly irrigation. This agrees with Gautam [1990], Ebrahimi et al. [2007] and Vahdati [2007].

Controlling temperature and RH in greenhouse improved grafting success compared to field condition or shade-house. High temperature in summer (Fig. 1) and low RH (Fig. 2) which reduced water availability for the outdoor grown plants reduced success of grafting especially in weekly irrigation in patch budding. These results are partially in line with the findings on walnut [Wani et al. 2017]. Wani et al. [2017] showed that the grafting success in walnut was significantly affected by grafting dates and conditions. Wani et al. [2017] reported that improved grafting success with maximum, 80.43% (in 30th January), and minimum, 41.4% (in 20th February), under greenhouse condition. Also under outdoor conditions maximum and minimum of grafting success was achieved in 21th March with 15.9% and 1th March with 11.8 %, respectively.

Ebrahimi et al. [2007] and Rezaee et al. [2008] reported that the lack of good success in grafting of walnuts could be attributed to the high temperature during grafting which is harmful to the growth of cal-

Table 6. The interaction between environmental condition × irrigation regime × grafting method on callus quality, graft-take, survival and scion height

Conditions of grafting/budding × irrigation regimes × grafting/budding method	Callus quality [A]	Graft/bud-take (%)	Survival (%)	Scion height (cm)
G × I × P	1.50^{f}	66.6^{bc}	27.7^{cde}	79.0^{d}
G × II × P	2.20^{b}	72.2^{b}	55.5^{ab}	81.3^{cd}
G × III × P	3.10^{a}	94.4^{a}	72.2^{a}	82.7^{cd}
G × I × T	1.80^{e}	44.4^{de}	38.9^{b-d}	90.0^{bcd}
G × II × T	1.90^{d}	55.5^{cd}	44.4^{bc}	95.0^{abcd}
G × III × T	2.00^{0c}	66.6^{bc}	44.4^{bc}	106.7^{abc}
S × I × P	1.20^{ij}	44.4^{de}	38.3^{bcd}	41.0^{f}
S × II × P	1.20^{ij}	55.5^{cd}	44.4^{bc}	45.0^{ef}
S × III × P	1.00^{k}	39.0^{ef}	27.7^{cde}	46^{ef}
S × I × T	1.30^{gh}	39.0^{ef}	27.7^{cde}	116.7^{a}
S × II × T	1.40^{g}	44.4^{ed}	38.8^{bcd}	117.3^{a}
S × III × T	1.21^{ij}	33.3^{ef}	27.7^{cde}	114^{ab}
O × I × P	0.80^{l}	11.1^{g}	11.1^{ef}	30.0^{f}
O × II × P	0.70^{l}	05.5^{g}	00.0^{f}	0.0^{g}
O × III × P	0.45^{m}	00.0^{g}	00.0^{f}	0.0^{g}
O × I × T	1.15^{kj}	33.3^{ef}	27.7^{cde}	111.7^{ab}
O × II × T	1.21^{ij}	27.7^{f}	22.2^{ed}	112.3^{ab}
O × III × T	1.00^{k}	27.7^{f}	16.6^{ef}	70.0^{ed}

G – greenhouse, S – shade-house, O – outdoor, P – patch budding, T – tongue grafting, I – irrigation regime daily, II – irrigation regime every three day, III – irrigation regime weekly

[A] Values are means of callus scoring from 1 (low callus) to 4 (very good callus)

Means with different letters in each column are significantly different according to DMRT at $P \leq 0.05$

lus. This agrees with our data (Tab. 6). Insufficient callus formation by scion and rootstock was found to be the main cause of graft failure in walnuts. Highly turgid cells are more likely to give proliferation of callus than those in a wilt condition [Wani et al. 2004, Dehghan et al. 2010]. *In vitro* studies, Doley and Leyton [1970] have shown that callus formation on cut surfaces reduced markedly with the decrease in water potential. The parenchyma cells comprising the callus tissue are thin walled and very tender. They hardly resist any desiccation and as such, if they are exposed to dry conditions for a long time, they would get killed [Wani 2004].

Ebrahimi et al. [2007], Avanzato and Atefi [1997] and Achim and Botu [2001] have reported a positive correlation between RH and temperature on grafting/budding success. Temperature between 24 and 28°C and RH between 80–95% is recommended for improving walnut grafting success [Millikan 1971, Avanzato and Atefi 1997, Suk et al. 2006, Dehghan et al. 2009]. Suitable temperature for grafting/budding under greenhouse conditions gives more success [Mir and Kumar 2011].

Finding a convenient and inexpensive outdoor condition to propagate grafted/budded walnut plants, was the main purpose of these experiments. Adequate temperature and RH showed an important role in improved graft success under shade-house versus outdoor (Tab. 1). In our experiment, shade-house tunnel, with fifty percent reduction of sunlight was used and

a mist system was applied to spray water from above the grafted seedlings every half an hour for thirty seconds from 9 a.m. to 19 p.m. for increasing the RH and decreasing the temperature.

The results revealed that shade-house with 42.6% graft success were better than outdoor conditions (17.6%) which agree with Vahdati [2007] and Mir and Kumar 2011]. Also graft-take with everyday irrigation in greenhouse using patch budding was 66.6% but reduced to 27.7% for graft survival, because of root-rot caused by saturated conditions of water in the pot.

Daily irrigation under outdoor conditions, resulted in higher graft success ratio compared to every three days and weekly intervals tongue grafting (33.3%, 27.7% and 27.7%, respectively) vs. patch budding (11.1%, 5.5% and 0%, respectively) which is in contrast with the result of Ramos [1985] and Vahdati [2007].

Ramos [1985] and Vahdati [2007] reported a positive correlation between temperature fluctuations, rainfall and irrigation before/after grafting on root pressure and negative correlation between root pressure and grafting success. The greatest concern and the determining timing of grafting is the presence or absence of bleeding. Root pressure is most likely to occur when soil water potentials are high and transpiration rates are low. Bleeding is worse when temperature fluctuates. If a very cold night is followed by a warm sunny day, bleeding will start by about 10 a.m. of that day. Data suggest that a flush of bleeding may start about 7 days after a temperature spike – a rapid increase in temperature followed by a rapid drop within a few days. Also, bleeding will be more severe if an irrigation or heavy rain occurs 2 weeks before or 2 weeks after grafting [Ramos 1985].

However, the higher root pressure (xylem bleeding), in some trees such as grapevine and walnut in early-Spring, correlated with increased mineral uptake and soil temperatures which cause decrease of callus formation or death of scion [Vahdati 2007]. Once transpiration rate is high, water is taken up rapidly into the leaves and evaporates to the atmosphere, so a positive pressure never develops in the xylem [Taiz and Zeiger 2002].

There are several methods for decreasing sap bleeding, as described by Kuniyuki and Forde [1985], Ramos [1985], Rezaee et al. [2008] and Dehghan et al. [2009]. For example, if rootstocks head back (2 to 3 inches above the grafting site) by 1–2 weeks earlier than the grafting time, to a large degree, future bleeding will occur from these cuts instead of the graft site. If seedlings have already been cut and bleeding starts again, the seedling cut again in the morning just 2 cm to provide a fresh cut. Another procedure is to make cuts in the rootstock through the bark and into the wood at several places below the graft site without completely girdling the rootstock. Another alternative is root pruning with plow for reduce of roof surface and decrease of sap. Therefore, in this study, for decreasing of root pressure before grafting, seedlings were transplanted in the second nursery. We attribute this success to the effective control of xylem bleeding by transplanting of seedlings in the second nursery and potting and as a result providing better RH content around the grafted walnut with every day irrigation versus 3 or 7 day on outdoor.

According to Vahdati [2007] excess bleeding caused the formation of anaerobic conditions at the graft site resulting in a decrease of graft survival. If the sap stays under the tape after grafting, the scion often dies. Also other methods such as side grafting and some other ideas were reported [Ramos 1985, Vahdati 2007, Rezaee et al. 2008, and Dehghan et al. 2009]. Normally, after early growth in spring, root pressure becomes low (middle of spring or summer) in patch budding. We observed low xylem bleeding in early-Spring, as we planted seedlings in the second nursery, in agreement with Vahdati [2007]. Most of the roots were cut and the levels of xylem bleeding were very low (in case of tongue grafting). With this method, root pressure (xylem bleeding) was not a factor in graft failure. Shorter irrigation intervals increase RH, decrease temperature and make an appropriate microclimate to improve grafting/budding success of grafted/budded plants. Optimum temperature and RH for callus formation is 24–28°C and 80–95%, respectively, in our experimental conditions.

Various methods of grafting showed significantly different responses to graft take success and survival (Tabs 2 to 6). These have been reported to give varying degrees of success in different walnut growing countries of the world [Achim and Botu 2001, Rezaee et al. 2008, and Mir and Kumar 2011]. These observations (Tabs 2 to 6) are in conformity with those of Rezaee

et al. [2008] who reported that best success of graft-take was recorded in patch budding when performed in early spring (average 2004 and 2005) with 46.66% versus whip graft (average 2004 and 2005) with 2.5%. However, our results revealed that for walnut grafted under outdoor conditions this may not be recommended (Tab. 6). The graft success plays a key role in the nursery economics. This was achieved in the use of a shade-house with appropriate irrigation regime (convenient, inexpensive, and available) for warm region. According to these results, an improved grafting success using shade-house and 3-day irrigation interval after grafting was developed. The shade-house and irrigation provided closer to optimum temperature and relative humidity as opposed to the outdoor conditions.

CONCLUSIONS

These results revealed that modifying micro-climate using a shade-house equipped by a mist system in warmer area under outdoor condition, improves grafting/budding success. Rapid growing of seedlings causes by appropriate irrigating regime causes rapid callus growth. Appropriate irrigation intervals under outdoor conditions increases RH and decreases temperature and make an appropriate microclimate which affects graft-take, callus quality and survival.

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