original research paper

Acta Agriculturae Serbica, Vol. XV, 30 (2010) 165-172

Acta Agriculturae Serbica
FACULTY OF AGRONOMY • ČAČAK •

Walnut grafting success as affected by stratification

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Abstract: The effect of air temperature and relative humidity inside a stratification room on walnut grafting success was investigated in this study. The whip-and-tongue grafting technique was used. One-year-old seedlings of local types of walnut (*Juglans regia* L.) and five cultivars and selections, including cv. Šeinovo (control), Ovčar, G-286, Elit and G-139 were used for grafting. Three stratification treatments were employed. The air temperature inside the stratification room in 2003 and 2004 ranged from 26-28°C and 28-29°C respectively. As for relative humidity, the values measured in both 2003 and 2004 were within the 60-70% range. The average percent graft take was 70.0 % in 2003 and 83.4 % in 2004. The highest average graft success of 86.0% in both years in all the tested cultivars and selections was obtained with the treatment involving dipping scions and graft unions into paraffin, stratification in sawdust up to the union and covering with polyethylene foil. Among the cultivars and selections tested, the graft-take success was highest in cv. Šeinovo (87.7%) and lowest in selection G-139 (67.9%).

Key words: walnut, seedling, cultivar and selection, grafting, stratification

Introduction

Walnut production in Serbia has not been adequate to meet the actual market demand for this fruit species, the reason being the decades-long practice of propagating walnuts by seed from natural populations dominated by walnut types that produce fruit of poor quality. With the aim to improve walnut production, systematic work has been undertaken on walnut grafting, but much later than in other fruit species. Grafting methods used in propagating other fruit

Received: 25 July 2010 / *Accepted*: 11 October 2010

species have not proven satisfactory in walnut. The best results under temperate continental climate conditions are attained by using the whip-and-tongue bench grafting technique. Grafting success is affected by choice of cultivar and selection, rootstock quality (Mišić 1983, Mitrović *et al*. 2008), time of scionwood collection (Mitrović 1995), date of grafting, grafting method, choice of substrate (Korać 1978), stratification (Mitrović and Blagojević 2002), and in particular by air temperature and relative humidity inside the stratification room. Air temperature and relative humidity are factors determining callus formation, degree and rate of callusing and, hence, wound healing rate, directly affecting the graft take percentage.

The objective of this study was to determine the most favourable stratification conditions with respect to air temperature and relative humidity for graft-take success in walnut.

Materials and Methods

The experimental study was conducted in the stratification room at the Fruit Research Institute, Čačak, during 2003 and 2004. One-year-old local walnut (*Juglans regia* L.) seedlings 8-15 mm in diameter were grafted with scions of five walnut cultivars and selections, including cv. Šeinovo (control), Ovčar, Elit, G-139 and G-286. Scionwood of the tested cultivar and selections was collected during winter dormancy from mother trees grown at the Fruit Research Institute.

The grafting operation was conducted on 4 April 2003 and 7 April 2004 using the whip-and-tongue technique, involving a sample of 30 grafted plants per treatment for each cultivar tested. A randomized block design (5 cultivars × 3 stratification treatments × 4 replications) was employed, giving a total of 1800 grafted rootstocks. Fresh conifer sawdust disinfected with 0.3% Wenturin was used as the stratification medium.

The experiment included three stratification treatments:

Treatment 1**.** Stratification without the use of paraffin by covering the grafts with conifer sawdust completely, i.e. up to the top of the scion;

Treatment 2**.** Dipping of scions and graft unions into paraffin (paraffin temperature being 60-70^{o}C) and their stratification in sawdust up to the top of the scion;

Treatment 3**.** Dipping of scions and graft unions into paraffin, and their stratification in sawdust up to the graft union and covering with polyethylene foil.

A central heating system was used to heat the stratification room. Air temperature and relative humidity inside the stratification room were measured and recorded using a thermohygrograph (Type 252, Wilh. Lambrecht KG Göttingen, Germany). The temperature of the substrate was measured using a soil depth thermometer (NTOS, Zagreb) having a measuring range of -20^{o}C do +50^{o}C.

The obtained results were subjected to Fisher's model of three-factorial analysis of variance – ANOVA (Fisher 1953). The significance of the differences between the means of the control cultivar and the other selections at $p \leq 0.01$ and $p \leq 0.05$ was defined through single and multiple comparison tests using Dunnett's test (Dunnett 1955). The LSD test was performed at $p \leq 0.05$ to test the significance of differences between stratification treatments and years and that of interaction means.

Results and Discussion

The air temperature inside the stratification room in 2003 (Table 1) ranged from 26-28°C. The said variation induced fluctuations in sawdust temperature. Namely, sawdust temperature in boxes containing non-paraffined, paraffined and foil-covered grafts was 23.0-25.5°C, 24.0-26.0°C and 25.0-27.0°C, respectively. As for 2004, no substantial variation in stratification room temperature was recorded, the temperature being 28-29°C. As a result, sawdust temperature was higher than in 2003, being 24.5-26.5°C, 25.5-27.0°C and 26.0-28.0°C in boxes containing non-paraffined, paraffined and foil-covered grafts, respectively. The relative humidity of air in the stratification room during 2003 and 2004 was within the 60-70% range. Air humidity variations did not have any significant effect on grafting success, as the deeper layers of sawdust did not dry out.

Tab. 1. Relative humidity (%) and temperatures (°C) inside the stratification room and sawdust in 2003 and 2004

Year	Relative humidity	Stratification room temperature	Sawdust temperature, Treatment 1	Sawdust temperature, Treatment 2	Sawdust temperature, Treatment 3
2003	60.0-70.0	26.0-28.0	23.0-25.5	24.0-26.0	25.0-27.0
2004	60.0-70.0	28.0-29.0	24.5-26.5	25.5-27.0	26.0-28.0

The variations in stratification room temperature and, hence, sawdust temperature in boxes containing grafted rootstocks induced variation in percent grafting success across cultivars and selections, as well as across stratification treatments and years (Table 2).

The control cultivar Šeinovo produced a highly significantly larger number of successful grafts as compared to the other selections tested. The percentage of successful graft takes in cv. Šeinovo was 82.6% in 2003 and 92.8% in 2004, and was followed by those of selections G-286 (75.8% in 2003 and 86.7% in 2004) and Ovčar (70.0% in 2003 and 84.7% in 2004), and selections Elit and G-139 as the least successful in both years of the study, their percentage of successful grafts being 63.6% and 58.1% in 2003, and 75.0% and 77.8% in 2004, respectively. The tendency observed in the number of unsuccessful grafts was found to be contrary to that reported for the successful graft takes.

As for stratification treatments, the one without the use of paraffin gave a highly significantly lower number of successful grafts (57.0% - 2003 and 73.5% - 2004) and a highly significantly larger number of failed grafts (43.0% - 2003 and 26.5% - 2004) as compared to the other two stratification treatments. The difference between the treatment involving dipping the grafts into paraffin and the one employing both paraffin and foil cover was also highly significant in terms of the number of successful graft takes (73.0% - 2003 and 84.7% - 2004) and number of failed grafts (27.0% - 2003 and 15.3% - 2004). The best results were obtained with the treatment involving paraffined and foil-covered grafts in terms of the percentage of successful takes (80.0% - 2003 and 92.0% - 2004) and that of failed grafts (20.0% - 2003 and 8.0% - 2004).

Tab. 2. Walnut grafting success at the end of stratification

		Number of successful grafts at the end of stratification (%)	Number of unsuccessful grafts at the end of stratification (%)
Cultivar (A)	Ovčar	77.3±0.91**	22.7±0.91**
	Elit	69.3±0.67**	30.7±0.67**
	G-139	67.9±1.08**	32.1±1.08**
	G-286	81.2±0.57**	18.8±0.57**
	Šeinovo	87.7±0.63	12.3±0.63
Treatment (B)	Without paraffin	65.2±0.70 c	34.7±0.70 a
	Paraffin	78.8±0.48 b	21.1±0.48 b
	Paraffin and foil	86.0±0.44 a	14.0±0.44 c
Year (C)	2003	70.0±0.57 b	30.0±0.57 a
	2004	83.4±0.41 a	16.6±0.41 b
ANOVA			
Cultivar (A)		**	**
Treatment (B)		**	**
Year (C)		**	**
A x B		*	*
A x C		**	**
B x C		ns	ns
A x B x C		**	**

- A, B and C represent cultivars, treatments and years, respectively.
- Asterisks in vertical columns denote significant differences between means at $p \leq 0.05$ and $p \leq 0.01$ according to Dunnett's test and ANOVA (F-test) results respectively; ns- non-significant
- Values designated with the same lowercase letters within the columns for treatment, year and interaction means are not significantly different at $p \leq 0.05$ according to the LSD test.

Sawdust temperature showed variations across different stratification treatments, being higher on average in the treatment involving the use of polyethylene foil cover, as compared to the other treatments. The foil prevented the escape of heat and moisture, thus ensuring temperature uniformity, reducing temperature variations and preventing desiccation of the substrate. Average sawdust temperature was found to be higher in the paraffined treatment than in the non-paraffined one. This was possibly due to the fact that paraffin, being an amorphous material used primarily to tightly secure the contact between the rootstock and scion, facilitated the union and accretion between the graft components, which was accompanied by the release of a greater amount of heat energy, in itself a characteristic of exothermic processes. Temperature fluctuations were highest in the treatment without the use of paraffin. Consequently, this treatment achieved the lowest grafting success, in view of the aim of stratification to provide the most favourable temperature and moisture within the substrate without any major fluctuations caused.

Graft-take success across years showed significant differences. In 2003, the average graft-take success was 70.0%, being highly significantly lower than in 2004

(83.4%). A contrary tendency was observed in the number of failed grafts across the years.

Different temperatures inside the heated room have been recommended as optimal by a number of national and international authors dealing with bench grafting of walnut under controlled environments. The resulting findings are, nevertheless, similar to the results of the present study in terms of grafting success at the end of stratification.

Bugarčić and Mitrović (1985) suggested that the production of grafted walnut plants is a highly complex technology, which often yields an unsatisfactory percentage of graft takes, ranging from 60.0-80.0%. Bulatović (1985) reports that an optimal stratification temperature of 25-27°C facilitates good callus formation at the graft union, with the grafting success being 65.0-90.0%. Stanisavljević and Mitrović (1997) obtained a graft-take success of 81.9% at a temperature of 26-28°C, in cv. Šeinovo (92.9%), selections G-286 (89.9%), Ovčar (89.4%), Elit (89.9%) and G-139 (82.0%). Mitrović *et al*. (2008) recommend stratification room temperature during the first 5-7 days to be maintained at 25-28°C until the substrate is heated, and then to be reduced and maintained at 24-25°C until the end of the stratification process. Korać (1978) obtained different graft take percentages at different temperatures used for walnut grafting. The graft take percentage at 26-28°C, 30-32°C and 22-24°C was 80.0%, 51.1% and 67.0%, respectively. The temperature of the heated room as recommended by Korać *et al*. (1997) should be 30-32°C during the first days after grafting, upon which it should be maintained at 27-28°C at the graft union. The same authors report the importance of much higher temperatures for walnut callusing than for other fruit species. In Slovenia, Solar *et al*. (2001) employed the omega bench grafting technique in selection Elit during the first half of April at 26-28°C and reported that indoor grafting results in 83.0% plants with callus formation. Karadeniz *et al*. (1997) report optimal stratification temperatures of 26°C (±2°C), but suggest that grafting success is also dependent upon grafting technique, air temperature and humidity, sap flow and other factors, resulting in 49.0-70.0% graft take. Long-term studies by Nedev *et al.* (1976) conducted in Bulgaria suggested that room temperature at the beginning of stratification should range from 18-20°C, but should be raised to 38-40°C for two to three days and, then, maintained at 25-28°C for a period of 10-12 days. The year-dependent grafting success as obtained by the said authors ranged from 77.9 to 93.4%. In Turkey, Barut (2001) recommends temperatures within the 22-29°C range. The grafting success in the first and second years was 75.0-90.0% and 83.0-95.0% respectively, depending on the cultivar used. Barut suggested that the results obtained served as a confirmation of the fact that environmental effects, primarily temperature, are a major factor governing walnut grafting as they directly facilitate callus formation. Ozkan and Gumus (2001) employed whip-and-tongue grafting at 27°C and achieved 30.0-70.0% graft-take success. Graft success was 63.0% in Romania as obtained by Achim and Botu (2001), 64.0% in Hungary by Lantos (1990), 55.3% in Poland by Porebski *et al.* (2002), 30.2-66.9% in Turkey by Ferhatoglu (1997), 58.24% in Turkey by Kazankaya *et al*. (1997) and 62.5% in Butan by Tshering *et al*. (2006).

Conclusions

The results obtained in this study suggest the following:

The observed differences in grafting success at the end of the stratification process are induced by different temperature regimes both within the stratification room and sawdust.

Grafting success was higher at the stratification room temperature of 28-29°C during 2004 than at 26-28°C in 2003.

The measured relative humidity variations of 60.0-70.0% in 2003 and 2004 did not have a significant effect on callus formation and graft success, as the variation did not induce desiccation in deep sawdust layers.

Grafting success at the end of stratification was found to be best in cv. Šeinovo (87.7%) and lowest in selection G-139 (67.9%).

The highest number of successful grafts (86.0%) at the end of stratification was obtained with the stratification treatment involving the use of paraffin and polythelene foil covered graft plants, followed by the paraffined treatment (78.8%) and non-paraffined treatment (65.2%).

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UTICAJ STRATIFIKOVANJA NA PRIJEM OKALEMLJENOG ORAHA

- originalni naučni rad -

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Rezime

U eksperimentu je praćen uticaj temperatura i relativne vlažnosti vazduha u stratifikali, na prijem okalemljenog oraha. Kalemljenje je obavljeno ručno, engleskim spajanjem na jezičak. Za kalemljenje su korišćeni jednogodišnji sejanci domaćeg oraha (*Juglans regia* L.) i pet sorti i selekcija oraha: Šeinovo (kontrola), Ovčar, G-286, Elit i G-139. Primenjene su tri varijante stratifikovanja. Temperatura u stratifikali 2003. godine kretala se od 26-28^{o}C, a 2004. godine od 28-29^{o}C. Relativna vlažnost vazduha u stratifikali tokom 2003. i 2004. godine varirala je od 60-70%. U 2003. godini prosečni prijem kalemova iznosio je 70,0% a u 2004. godini 83,4%. Prosečno najviši prijem kalemova od 86,0% u obe godine ispitivanja kod svih sorti i selekcija utvrđen je kod varijante sa parafinisanjem plemke i spojnog mesta, stratifikovani strugotinom do spojnog mesta i prekrivani polietilenskom folijom. Kod ispitivanih sorti i selekcija najbolji prijem kalemova imala je sorta Šeinovo (87,7%), a najmanji selekcija G-139 (67,9%).