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**FIRST RESULTS OF INDUSTRIAL PROPAGATION OF WALNUT (*J. REGIA* L.) IN BULGARIA BY THE HOT CALLUS METHOD, USING HOT WATER INSTALLATION**

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**Abstract**

The experiment was carried out at the Fruit-Growing Institute – Plovdiv, Bulgaria. The principles of the hot callus method of walnut propagation were applied. A hot water installation was built for maintaining a temperature of 27°C ( $\pm$  1°C) at the place of grafting. The installation comprised of a boiler, a pump, metal tunnels with doors, soil heating pipes, valves, etc.

The principles of the installation and the elements of the technological process were described. The results showed that successfully propagated walnut plants were obtained by applying the hot water installation. The percentage of the successfully propagated plants was 70.0% when cool-stored scions were used and 82.0% when using unstored scions. It was concluded that the hot water installation could be applied in practice.

**Key words:** walnut(*J. regia* L.), propagation, hot callus, hot water installation.

**Introduction**

Propagation of walnut(*J. regia* L.) is more difficult compared to most of the fruit species. It is due to the low rate of callus formation and the presence of high concentration of phenolic compounds (Rongting & Pinghai,1993). Because of walnut heterozygosity, propagation by seeds does not lead to inheritance of the characteristics of a certain variety. For that reason, different methods of walnut propagation have been investigated all around the world. Propagation with cuttings is difficult (Gautam,1990) and at present budding and grafting are the most popular inoculation techniques in the production of walnut trees (Gandev, 2007).

Patch budding is most commonly used in walnut propagation. This is one of the oldest and popular techniques for propagation in a nursery in the open (Kuniyuki & Forde, 1985). Scientific literature reported different data on the efficiency of this inoculation method (Dzhuvinov et al., 2010).

In the recent years, local heating of the graft union with a hot callusing pipe was successfully applied (Lagerstedt,1981; Avanzato, 1997; Avanzato & Tamponi, 1988; Gandev, 2009). The trial methods for local heating of the graft union have been different in the different experiments but the purpose is the same – to achieve a temperature of 26-27°C. According to Lagerstedt (1979), Millikan (1984) and Wilbur et al. (1998), the optimal temperature for walnut callus formation is 26-27°C.

The aim of the present investigation was to create, test and evaluate the hot water installation for walnut propagation under production condition.

## Materials and methods

### Principles of the hot water installation for grafted walnut trees

The experiment for industrial propagation of walnut (*J. regia* L.) by the hot callus method was carried out in 2013 at the Fruit Growing Institute – Plovdiv. A hot water installation for grafted plants was built indoors and tested for its efficiency. The installation is a close water system, in which the circulating hot water maintains a permanent temperature in the heated tunnels. The distance between the separate tunnels is 1.2 m. The elements of the installation comprise of a boiler, a pump, metal tunnels with doors, soil heating pipes placed in the tunnels on perlite, valves and fasteners.

Water in the boilers was maintained at 50°C ( $\pm 1^\circ\text{C}$ ). The temperature in each tunnel depended on the quantity of the water flowing through the soil heating pipe placed at the bottom of the tunnel. Water flow in the tunnels was controlled by valves.

### Walnut Grafting

520 walnut plants of ‘Izvor 10’ cultivar were grafted in the period 11-13 March. Two types of scions were used in both variants of the trial:

First Variant – scions stored for three months in a refrigerator at a temperature of 2-5°C and

Second Variant – scions collected and grafted on the same day.

Cleft grafting was carried out. During the grafting procedure, the vascular cambium of the scion and the rootstock were adhered to each other. The graft was fixed with a rubber cord, which was not tight, in order to allow the exit of the surplus moisture at the place of grafting. According to Rongting & Pinghai (1993) excess bleeding caused the formation of anaerobic conditions at the graft juncture, resulting in a decrease of the graft survival rate. One year old seedlings of *Juglans regia* L. were used as rootstocks. Scions were kept in water for one day to increase the moisture content before grafting. The root tip of each rootstock was cut in order to stimulate the formation of new lateral rootlets. The scion tops were immersed in warm paraffin to prevent water loss (Fig. 1)



Fig. 1. Grafted plants before placing for callus formation.

The grafted plants were laid horizontally over the tunnels, transversely to the longitudinal axis of the tunnel, the place of grafting being exactly at the heated point. The roots of the plants,

which were outside the tunnel, were covered with wet sand. The tunnel doors were tightly closed and covered with plastic folio to decrease moisture evaporation (Fig. 2).



Fig.2 .Heated tunnels stocked with grafted plants.

#### Caring for the grafted plants

Heating was provided for the grafted plants for four weeks, the temperature of  $27^{\circ}\text{C}$  ( $\pm 1^{\circ}\text{C}$ ) being maintained at the place of grafting. The air temperature in the premise where the installation was built, was maintained within  $10$  to  $18^{\circ}\text{C}$  by a convector. Plant roots were periodically wetted every few days. When necessary, the perlite in the tunnel was also wetted to provide uniform moisture levels at the place of grafting. In cases of rootstock suckering, the suckers were immediately removed. At the end of the fourth week, when the process of callus formation finished, the heating installation was switched off.

Successfully grafted plants (Fig. 3) were potted and adapted for a month (Fig. 4). At the end of the period of adaptation, the percentage of the successfully propagated trees was reported.



Fig. 3.Plants after callus formation.



Fig. 4. Potted plants.

The experimental design was a complete randomization with five replications per treatment and 20 grafts in each replication.

The data obtained were statistically processed by Duncan's test (Steele & Torrie, 1980).

#### Results and discussion

Obtained results showed that the use of the constructed hot water installation contributed to the success of walnut propagation (Tabl. 1). Throughout the heating period the reported temperature in the tunnels was  $27^{\circ}\text{C}$  ( $\pm 1^{\circ}\text{C}$ ), which showed the reliability of the installation and as an important precondition for successful callus formation in the grafted plants (PaunoviC et al., 2010).

**Tabl. 1.** Percentage of successfully propagated plants

<b>Variants</b>	<b>Percentage of successfully propagated plants</b>
1. Unstored scions	82.0 a
2. Cool-stored scions	70.0 b

Statistically different at  $P=5\%$

Under the climatic conditions of Bulgaria, the buds of the mother walnut trees suffer severe frost bites quite often (Gandev, 2012), which sometimes compromises winter grafting of walnut. The results obtained in the carried out experiment showed that in such years the scions collected before the winter frosts, could guarantee a good percentage of successful grafting. Data presented in Tabl. 1 showed that when using scions collected immediately before grafting, the graft survival rate was 82.0% (Var. 1), while when using stored scions (Var.2), it was lower – 70.0%, the difference being statistically significant.

In the process of the study a certain cultivar-specific variation was established concerning the graft survival percentage, which could be explained by the varietal differences. Concrete data were not presented at that stage of the study. It was only established that among the three

grafted cultivars, i.e. ‘Izvor 10’, ‘Sheynovo’ and ‘Silistrenski’, the highest survival percentage was reported for the first cultivar.

### Conclusion

The first results of the carried out study on industrial walnut propagation by the hot callus method, using a hot water installation for heating the place of grafting, gave the grounds to draw the following conclusions:

The described hot water installation contributes to the production of successfully propagated walnut plants;

Walnut propagation is more efficient when using scions collected on the day of grafting compared to scions collected before and stored in a refrigerator.

### References

- Avanzato, D., 1997. Walnut graft-union callused by heating cable. Combined Proceedings-International Plant Propagator’s Society, 47: 211-214.
- Avanzato, D. and G.Tamponi, 1988. The effect of heating of walnut graft unions on grafting success. *Acta Horticulturae*, 227: 79-83.
- Dzhuvinov, V., S. Gandev, K. Koumanov and V. Arnaudov, 2010. Walnut. Biofruit BG, Plovdiv.
- Gandev, S., 2007. Budding and grafting of the walnut (*J. regia* L.) and their effectiveness in Bulgaria. *Bulgarian Journal of Agricultural Science*, 13: 683-689.
- Gandev, S., 2009. Propagation of walnut under controlled temperature by the methods of omega bench grafting, hot callus and epicotyl grafting. *Bulgarian Journal of Agricultural Science*, 15 (2): 105-108.
- Gandev, S. 2012. Winter hardiness of reproductive organs of the walnut cultivars Izvor 10, Lara and Fernor at extreme low temperatures in South Bulgaria. *Bulgarian Journal of Agricultural Science*, (in print).
- Gautam, D.R., 1990. Studies on the winter and summer vegetative propagation techniques of walnut (*J. regia* L.). *Acta Horticulturae*, 284:27-31.
- Kuniyuki, A. and H. Forde, 1985. Walnut propagation, pp. 38-46. In: Ramos, D. (Ed.), Walnut orchard management. Publication 21410, University of California, USA.
- Lagerstedt, H.B., 1979. Propagation-Seed, Grafting, Budding. In: Nut Tree Culture in North America, R. A. Jaynes (ed.). NNGA Broken Arrow, Road Hamden, Conn., 065518: 240-271.
- Lagerstedt, H. B., 1981. A new device for hot callusing graft unions. *HortScience*, 16:529-530.
- Millikan, D.F., 1984. Propagation of *Juglans* Species by Fall Grafting. Annual Report of the Northern Nut Growers Association, 61: 41-44.
- PaunoviC, S., M. MitroviC, R. Miletic, 2010. Callus formation as affected by air temperature under different variants of walnut stratification. *Journal of Pomology*, 44: 123-128.
- Rongting, X. and D. Pinghai, 1993. A study on the uniting processes of walnut grafting and the factor affecting. *Acta Horticulturae*, 311: 160-170.
- Steele, R. and Torrie, J., 1980. Principles and procedures of statistics. McGraw-Hill, New York.
- Wilbur, O.R., C.A. Leslie, H.I. Forde and J.R. McKenna, 1998. Propagation. In: D. E. Ramos (ed.). Walnut production manual. University of California, Division of Agriculture and natural resources, Publication 3373.