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## **PROPAGATION OF WALNUT (*JUGLANS REGIA* L.) UNDER CONTROLLED TEMPERATURE BY THE METHODS OF OMEGA BENCH GRAFTING, HOT CALLUS AND EPICOTYL GRAFTING**

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### **Abstract**

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The basic way for walnut propagation in Bulgaria is the Patch budding method. The grafted plants are grown in the open in the nursery and during years with low winter temperatures or spring frosts the survival rate is low. The aim of the experiment was to study and compare different methods of walnut propagation, in which the grafting success does not depend on the external climatic conditions. The investigation was carried out in 2006-2007 with cultivar 'Izvor 10' grafted on common walnut rootstock (*Juglans regia* L.). In the two years of the experiment at the end of March the tested grafted plants were placed under controlled temperature of 27°Ñ for callus formation. Four weeks after grafting the plants were transferred to a room temperature of 15-18°Ñ for twenty days, after which the percentage of graft take was recorded. The lowest percentage of successfully grafted plants was reported for the classical Omega bench grafting method (control) – 40.8 % in both years of the experiment. For the new propagation techniques, close percentages of successfully grafted plants were obtained – 74.2 % in average for the method of Hot callus and 77.5 % for the method of Epicotyl grafting. As a conclusion, the use of the new methods of walnut propagation – Hot callus and Epicotyl grafting – increased the percentage of the successfully grafted plants in the period of winter dormancy under controlled temperature.

*Key words:* walnut, *Juglans regia* L., grafting, omega bench, hot callus, epicotyl grafting.

### **Introduction**

The basic way for walnut propagation in Bulgaria is the Patch budding method when the grafted plants are grown in the open in the nursery (Nedev et al., 1976). The disadvantage of that method is the fact that during years with low winter temperatures or spring frosts the grafted buds are exposed to unfavorable climatic conditions. The last four years at the Fruit-Growing Institute - Plovdiv the percentage of

successfully propagated plants by the patch budding method in the open varied within 33 % to 47 % (unpublished data). Walnut propagation under controlled temperature eliminated the unfavorable effect of the external climatic conditions; however, the different methods under controlled temperature were characterized by different percentages of graft success. In the classical method of Omega bench grafting (Nedev et al., 1976) and in the new methods of Hot callus grafting (Avanzato, 1997; Avanzato, Tamponi, 1988;

Avanzato et al., 2006) and Epicotyl grafting (Suk-In et al., 2006; Gandev and Arnaudov, in print) the percentage of survival varied within the limits of 7 to 100 %. Comparisons among those methods are difficult due to the fact that the separate experiments have been carried out with different cultivars and rootstocks.

The aim of the present investigation was to study and compare the walnut propagation methods Omega bench grafting, Hot callus and Epicotyl grafting.

## Materials and Methods

The investigation was carried out in 2006-2007 with cultivar 'Izvor 10' grafted on common walnut rootstock (*Juglans regia* L.). In the two years of the experiment at the end of March the tested grafted plants were placed under controlled temperature of 27<sup>o</sup> C for callus formation. Four weeks after grafting the plants were transferred to a room temperature of 15-18<sup>o</sup> C for twenty days, after which the percentage of grafting was reported.

Three methods of propagation were compared, as follows:

1. Omega bench grafting (the classical method). Omega grafting was conducted using one-year old rootstocks and scions with a grafting machine (Figure 1). Graft unions were plunged into hot paraffin (70-80<sup>o</sup> C) and then cooled in cold water. The graft unions were forced in woody boxes, filled with wet sawdust in a room under controlled conditions at 27<sup>o</sup> C and 80 % relative humidity (Nedev et al., 1976) .

2. Hot callus method. The experiment was set in

an unheated green-house. Cleft grafting was carried out with one-year old rootstocks and scions. The grafted plants were tied with a plastic band and the grafting place was covered with foil. Thus prepared, the plants were placed horizontally, the place of grafting being located above an electric heating cable buried in a plastic U-shaped pipe filled with peat (Figure 2). After that their roots were covered with wet sand and wet foam was put over the place of grafting, pressed with weights to the plastic pipe in order to save the heat and to maintain the temperature of 27<sup>o</sup> C at the place of grafting. Periodically the roots were watered and the foam was wetted (Avanzato et al., 2006) .

3. Epicotyl grafting method. Cleft grafting was conducted with one-year old scions on 10 to 15-day-old new shoots of germinating the walnut fruit. Scion thickness corresponded to the thickness of the new shoot of the rootstock. Polyethylene band was used for tying to provide good aeration at the place of grafting (Figure 3). Thus prepared the plants were placed horizontally one to the other in plastic troughs and their roots were covered with a wet mixture of equal parts of sawdust and perlite. A polyethylene band was put to protect the place of grafting from moistening by the wet mixture. After the trough was filled with the grafted plants, it was tightly covered with polyethylene to prevent from airflow and it was placed in a room at a temperature of 27<sup>o</sup> C. After 12-16 days the grafted plants started developing new shoots. When the new shoot of grafted plant reached the length of 4-5 cm, the plant was taken out of the trough and it was pot-



**Fig. 1. Omega bench grafting plant**



**Fig. 2. Hot callus method - grafted plants placed for heating over an electric cable**



**Fig. 3. Epicotyl grafting method – One-year old cutting grafted on ten-day old shoot germinated from the walnut fruit**



**Fig. 4. Epicotyl grafting method – potted plants**

ted (Figure 4), while the rest of the plants remained in the trough to continue the callus formation under controlled temperature conditions (Suk-In et al., 2006; Gandev and Arnaudov, in print).

Every year each of the variants was represented by 60 plants. The data were statistically analyzed by Duncan's test (Steele and Torrie, 1980).

## Results and Discussion

The results presented in Table 1 showed that in both years of the experiment the grafting rate was affected by the different methods of grafting. The lowest percentage of survival was reported in the plants propagated by the method of Omega bench grafting – 40.8 % in average for the two experimental years. It should be noted that the results obtained when applying that method differed in the two experimental years. In the first year the successfully grafted plants were 50.0 % and in the second one – only 31.7 %. Solar et al. (2001) also mentioned about similar variation of the grafting rate in the separate years when that method was applied. Using the same method of walnut propagation with the only difference that cleft and whip-tongue grafting was applied, Ozkan and Gumus (2001) established that the percentage of survival depended on the month when grafting was carried out and on the grafted cultivar. In February for cultivar 'Yalova 1' the authors obtained the highest percentage of successfully propagated plants – 63 % when applying cleft grafting and 70 % for whip-tongue

grafting, while in January for cultivar 'Tokat' only 30 % of successfully propagated plants were obtained from both methods applied. In our experiment a higher percentage of successfully propagated plants was obtained from Hot callus and Epicotyl grafting methods in comparison with the classical Omega bench grafting method. With the Hot callus method 76.7 % of successfully grafted plants were obtained in the first year and 71.7 % in the second one. Results obtained from Epicotyl grafting were similar – 80.0 % in 2006 and 75.0 % in 2007. In both years 74.2 % of successfully propagated plants were obtained in average from the Hot callus method and 77.5 % from the Epicotyl grafting method. For Epicotyl grafting method Suk-In et al. (2006) announced that the survival rate was from 65.0 % to 85.5% depending on the time of propagation. The best results were obtained in February.

When applying the Hot callus method in March, Avanzato et al. (2006) announced that they obtained different percentage of successfully propagated plants of selected walnut hybrids, which was due to their different genotypes. According to Pinghai and Rongting (1993) the differences among the genotypes were explained by their chemical constituents. The better ripeness and quality of the scions, which contained higher soluble sugars, starch and C/N ratio, the higher the grafting survival percentage was. In our experiment grafting was carried out only with cultivar 'Izvor 10' in one and the same month (in February) in both years of the experiment. That enabled us to compare

**Table 1**  
**Percentage of grafting success in cultivar 'Izvor 10' propagated by Omega bench grafting, Hot callus and Epicotyl grafting methods**

Grafting methods	Year		Average
	2006	2007	
Omega bench grafting	50.0 b	31.7b	40.8 b
Hot callus	76.7 a	71.7 a	74.2 a
Epicotyl grafting	80.0 a	75.0 a	77.5 a

the methods used for walnut propagation – Omega bench grafting, Hot callus and Epicotyl grafting – and to evaluate their efficiency.

## Conclusion

The application of the Hot callus and Epicotyl grafting methods increased the graft success compared to the grafted plants by the Omega bench grafting method.

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