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New technology (in situ grafting) for faster production of walnut (Juglans regia L.)

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Abstract: Technology regarding grafted walnut production is very complex and expensive because it depends on a number of factors that directly influence grafting success. Due to a long production period and a smaller number of first class plants compared to other fruit species, young walnut trees are among the most expensive. New in situ production technology of young walnut trees has led to quicker production, shorter by 1 year, and increased the success of grafting, allowing for large-scale production of grafted walnut. In order to increase the production of quality planting material for walnut varieties, the possibility of walnut grafting in the open, i.e. in situ, was examined herein. Based on the average results for all of the varieties/selections, similar performance was achieved with grafting (57.14%) and the number of first class plants (55.71%) when compared to conventional grafting (54.46% and 53.39%, respectively), but it was concluded that this method shortened the process of plant production for 1 vegetation. The greatest success with the application of in situ grafting was with the Rasna selection, which had significantly the best grafting take (72.86%) in comparison with the other walnut varieties examined during the research period. By comparing the success of the indoor and in situ production methods and examining the influence of certain factors on production success, it was concluded that the in situ method proved to be a better option for a simpler, more profitable, and faster mass production of high-quality walnut planting material.

Key words: In situ grafting method, walnut, young plant trees

1. Introduction

Fruit species contribute to world biodiversity through numerous cultivars, genotypes, and accessions. Fruit cultivation has been practiced in most countries around the world for centuries, for nutrition and as an income source. They also meet personal and social needs such as curing diseases, beautifying the planet, etc. (Ercisli et al., 2003; Vijayan et al., 2008; Serce et al., 2010).

Walnut is one of the oldest plants distributed worldwide, ranking third in nut production (Fernandez-Lopez et al., 2000; Ercisli et al., 2012), and is considered to be quite healthy for human nutrition (Khir et al., 2014). It is used not only as an individual foodstuff, but also as an ingredient in several baked and processed foods (Eliseeva et al., 2017), depending on the fruit quality parameters (Warmund, 2008; Fuentealba et al., 2017; Demir, 2018). Amin et al. (2017) indicated that walnuts can be useful as potential natural antibacterial agents.

Creating fruit plantations represents a very costly long-term investment, where the most important goal is, on the one hand, cutting production costs, and, on the other, intensifying production. One of the main preconditions for the successful growing of fruits is setting up plantations with high-quality planting material.

Walnut grafting began much later than the grafting of other fruit species. Paladius (4th century) was the first writer to mention walnut grafting, and the results of walnut ring grafting were first provided by Moneceau (1763) and Roi (1772) (Schneiders, 1947). Although walnut has been grafted for more than 2 centuries, it is still not grafted in large proportions, since many orchardists who have successfully dealt with the propagation of many other fruit crops have not yet mastered the technique of walnut grafting, which is undoubtedly more complex.

Apart from the usual factors that influence the production of planting material for other fruits, production success and walnut plant quality are greatly affected by the grafting method (Avanzato and Tamponi, 1988; Porebski et al., 2002; Avanzato et al., 2006; Gandev, 2009; Soleimani et al., 2010; Dehghan et al., 2010a), when the grafting is done (Özkan and Gümüs, 2001; Bayazit et al., 2005; Suk-In et al., 2005), as well as the climate conditions of the region where the grafting takes place (Vahdati, 2003; Karadeniz, 2005; Wani et al., 2017). Of all of the rootstocks for walnut grafting, the domestic population of walnut (*Juglans regia*

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L.) has proven to be the best, especially in a continental climate (Nedev et al., 1976). In our climate, the best results have been achieved by manually grafting walnut onto a population of walnut *Juglans regia* L., by applying the English grafting method (Korać, 1978).

In Serbia, the entire production of walnut planting material has been done indoors, i.e. under room conditions, since it has been considered that walnut cannot be grafted successfully in the open in this climate. The biggest problem with the production of planting material has so far been the poor success of the grafts placed in stratification pits. Hence, the number of grafts taken to a nursery field is decreased by 20%–40% of the total number of those that were grafted, which presents a great loss. Apart from this, with the standard production of the planting material done via the indoor grafting method, several necessary operations must be done in order to produce the graft. With every step, the success rate decreases. As a result, there are no more than 30%–50% of the first class grafted plants from the total number of grafted rootstocks.

Since walnut planting material production is complicated, specific, and expensive, only a small number of nurserymen opt for its production. Due to the low production of high-quality planting material and the high cost of walnut plants, the number of new nurseries is decreasing year after year.

In order to improve walnut planting material production, the purpose of this research was to examine and implement an entirely new technology, i.e. to switch from the current method of indoor grafting to the production of walnut plants in the open (in situ), by which not only were the grafting success and quality of the planting material increased, but the production period was shortened from 2 years to 1 year.

2. Materials and methods

This research included 3 walnut varieties (Jupiter, Geisenheim 251, and Šampion) and 1 walnut selection (Rasna), which was grafted in 2 ways (indoor grafting and in situ grafting) on a 1-year generative rootstock of *Juglans regia* L. over a period of 2 years (2015–2016) in the Bogdanović nursery in Temerin, Vojvodina (Serbia). The selection of the investigated varieties and selection was based on the representation of the same in plantations in Vojvodina and its surroundings.

The land conditions in the nursery in Temerin were very suitable, due to the type of chernozem present there. The level of groundwater was favorable. Vojvodina is a region under the influence of a continental climate. The mean annual temperature is about 11 °C. High summer and low winter temperatures are characteristic. The mean annual cloudiness is about 45%. Vojvodina has a continental rainfall regime (550–750 mm) and the vegetation period is characterized by dry and rainy periods of different durations and intensity, with more rain in May and June, and then slightly less in July and August. In the warm part of the year, there is plenty of summer rain, and occasionally hail, but the snow cover in winter is not thick, and often in winter there is no snow. Climatic conditions during the examined years were in line with the average values of climate parameters for the region.

Scions were obtained from the mother trees of the walnut varieties and selections from the collection orchard in the same nursery, just before the beginning of vegetation in the examination years.

The research included 1120 pieces of generative rootstocks (*Juglans regia* L.) grafted with 3 varieties and 1 walnut selection. One half of the rootstocks (560 pieces) were grafted and nurtured according to the standard indoor grafting conditions, whereas the other half were grafted in the open, according to the new in situ method, which was the subject of this research. The English grafting method was applied for both methods of walnut planting material production. The quality of the walnut plants was determined by their health condition, the quality of the graft union, and development of the root system and the above ground part. The first class included plants with a well-developed root system (at least 5 basic vessels of 20 cm in length), that were normally developed, upright, healthy, and had a well-adhered graft union, over 50 cm tall, and diameter directly above the root door over 10 mm.

2.1. Indoor grafting

With the indoor method of production, the produced walnut rootstocks were taken out, prepared for grafting, grafted, and placed in a stratification pit to allow the scion and stock to form callus. During stratification for next 25 days, the relative humidity of the air was maintained at 70%–90%, while the temperature, which must be constant at the height of the graft union, was 27–28 °C. At the end of April, or the beginning of May, depending on the year, the successful grafts were taken out into the nursery field, where they were nurtured until the end of the following year.

2.2. In situ grafting

In situ grafting is a tunnel type of plant propagation, which represents a new method of walnut planting material production. It requires partial control of the temperature, which has a significant influence on grafting success. After grafting the 1-year rootstock of *Juglans regia* L. in the open, the union of the stock and scion were tied using the grafting foil, and the scion was coated with grafting wax. The coating was done in order to prevent the scion from drying and for the grafts to succeed. Grafting should be done at an atmospheric temperature of about 25 °C.

After the grafting had been done (April 24th, 2015 and April 20th, 2016), a framework for the foil, with dimensions of 50 × 50 × 25 cm, was set up. The frames were placed every 1.5 m for better stability and, therefore, better quality of the framework. The height of the frames was about 25 cm above ground. After the frames had been set up, a rope was stretched from the beginning to the end of the framework, on both sides, in 2 zones. The upper rope was for fixing the foil, and the transversal rope was to make the foil stable on the framework. The transversal rope was 120 cm long, while the length of the upper rope depended on the length of the tunnel. The foil used for building the tunnel was transparent, thin, and easily stretchable. The transparency of the foil was quite relevant because of the amount of sun light that must reach the plant. It provided constant temperature for the plant, since in the period of grafting, the temperature still varied during the day and, especially at night. During the day, when the daily temperature rose above 27 °C, the frames needed to be lifted (extracted from the ground), in order to decrease the temperature in the tunnels. The grafts had to be supervised daily, because of potential damage on the tunnels, as well as the appearance of new unruly sprouts, which grow from the stock. They needed to be timely removed in that way enabled the young plant to grow undisturbed. After removal of the tunnel, there were no plant losses due to adaptation to the external environment. The entire foil framework remained in the seedbed until the young plants were advanced enough and needed to be provided with more space for further growth and development (around the end of May). By the end of May, there was no oscillation in the temperature and the plants could grow freely. After the framework was removed, support, in the form of a metal bar, was placed next to each plant. They were first fixed to the bars with vineyard binder. The cleaning of the rootstocks (the removal of unruly sprouts) continued. Binding was repeated every 10 days in accordance with the growth of the plants. Apart from the removal of unruly sprouts, the movement of lateral branches was controlled, since they were most commonly activated on about 10% of the grafted rootstocks. Throughout the year, the soil was cultivated 5–6 times, as needed, and the seedbed was irrigated 2–3 times. The plants were treated with plant protection products 2–3 times, as needed, most commonly against dark stains on the leaves.

In autumn, after the leaves fell off, i.e. after defoliation of the planting material, the plants were removed with a plough.

The obtained data of the production success of the planting material for the walnut varieties and selection were analyzed using the factorial analysis of the variance in the statistical program STATISTIKA (www.statsoft.com). The significance of the difference between the means of the examined characteristics was tested using the Duncan test for significance at $P < 0.01$. The results are presented in the Tables for the average value for the examination period.

3. Results and discussion

By comparing the standard indoor method of walnut plant production and the new in situ grafting method, depending on the variety/selection and the year of examination, different growth success was achieved. At the same time, there was a difference where the indoor method enabled the production of the first class plants, which were put up for sale 2 years after grafting, whereas the first class plants were produced by the in situ method in a 1-year period, and put up for sale in the autumn of the same year, after defoliation. What could be noticed, on average, for the examination period, was a statistically significant influence of the examined variety/selection on the success of the walnut plant production by both grafting methods (Table 1). The greatest indoor success was achieved with both the Rasna selection and Šampion variety (64.30%), which were statistically much better in comparison with varieties G 251 (47.14%) and Jupiter (42.14%). The average success rate with the indoor method was 54.46%, where a total of 305 walnut plants were produced from 560 grafted rootstocks. A comparison of the grafting success of different cultivars showed that the highest success and the lowest losses (70.8% and 22.2%, respectively) were detected with the Chandler cultivar (Gandev and Arnaudov, 2011). Other researchers have also cited similar grafting success, depending on the variety (Rongting and Pinghai, 1990; Avanzato et al., 2006), grafting conditions (Vani et al., 2017; Karadeniz, 2005), and grafting method (Solar et al., 2001; Gandev, 2009).

The greatest in situ success was achieved with the Rasna selection (72.86%), while quite lower, yet similar, production success was noted with varieties G 251 (57.86%) and Šampion (57.14%). The lowest relevant production success with the in situ method was seen with the Jupiter variety (40.71%).

The average success rate for the in situ production of the grafted planting material throughout the research period was 57.14%, where 320 walnut plants were produced from the total of 560 grafted rootstocks.

There was no record of any statistically significant difference in the production success depending on the grafting method for the varieties/selections examined throughout the research period. Namely, the success of the walnut plant production was steady, with the difference that with the application of the in situ method (57.14%), only 1 vegetation was needed for the production of the plants, while it took 2 vegetations for the production of the same amount (54.46%) by means of indoor grafting.

The following 2 tables show the phases, duration, and the differences in the production of plants between the indoor grafting method (Table 2) and in situ walnut grafting method (Table 3).

During 2015–2016, applying the indoor method, 560 stocks in total were grafted with all of the varieties

Table 1. Production success of the walnut planting material depending on the variety/selection and the grafting method applied (2015–2016).

Variety/selection	No. of grafted rootstocks	No. of plants produced	Production success (%)
Indoor grafting			
Rasna	140	90	64.30 ab
Šampion	140	90	64.30 ab
G 251	140	66	47.14 bc
Jupiter	140	59	42.14 c
Total/ average	560	305	54.46 A
In situ grafting			
Rasna	140	102	72.86 a
Šampion	140	80	57.14 bc
G 251	140	81	57.86 bc
Jupiter	140	57	40.71 c
Total/ average	560	320	57.14 A

* Values in the same column with different letters were significantly different according to the Duncan test (P < 0.01).

Table 2. Production of first class walnut plants by indoor grafting after the first and the second production year (2015–2016).

<table>
<tr><th rowspan="2">Variety/selection</th><th rowspan="2">No. of grafted rootstocks</th><th rowspan="2">Planted in the field compared to the grafted plants (%)</th><th colspan="2">No. of plants in the field after the first production year</th><th colspan="2">No. of plants in the field after the second production year</th></tr>
<tr><th>Compared to those planted in the field (%)</th><th>No. of first class plants</th><th>Compared to the grafted plants (%)</th><th>First class plants (%)</th></tr>
<tr><td>Rasna</td><td>140</td><td>82.14</td><td>83.48</td><td rowspan="5">There were no grafted plants produced that could be put up for sale after the first year of production</td><td>64.29</td><td>64.29 a</td></tr>
<tr><td>Šampion</td><td>140</td><td>76.43</td><td>85.05</td><td>64.29</td><td>63.57 a</td></tr>
<tr><td>G 251</td><td>140</td><td>80.00</td><td>69.64</td><td>47.14</td><td>45.00 b</td></tr>
<tr><td>Jupiter</td><td>140</td><td>75.71</td><td>65.09</td><td>42.14</td><td>40.71 b</td></tr>
<tr><td>Total/average</td><td>560</td><td>78.57</td><td>75.82</td><td>54.46</td><td>53.39</td></tr>
</table>

* Values in the same column with different letters were significantly different according to the Duncan test (P < 0.01).

and selections examined, 440 of which were taken out of the stratification pits (78.57% of those that had been grafted). At the end of the first year, there were no plants that could be put up for sale (Table 2). After the first year, there were 334 walnut grafts remaining in the nursery field (75.82% of those that had been grafted). After the second year of growing, there were 305 plants remaining in the nursery field (90.69% of those counted in the field after the first year, i.e. 54.46% of those that had been grafted). The success of the first class walnut plant production compared to the total number of rootstocks grafted on average, for all of the varieties and selections examined throughout 2015–2016, was 53.39% (299 plants produced). Karadeniz (2005) stated that the graft-take success varied from 29% to 64%, depending on the year, while Dehghan et al. (2010b) noticed that grafting success varied from 24.31% to 82.22%, depending on the grafting method used. According to the results of other researchers, graft-take success usually varied in different walnut varieties and selections (Kuden and Kaska, 1997; Stanisavljevic and Mitrovic, 1997).

On average, for the given period, out of the 560 stocks grafted with the varieties and selections examined by in situ method (Table 3), after the first year of production, 320 plants were left in the nursery field (57.14% of those that had been grafted). The success of in situ production of first class walnut plants for the varieties/selections examined was, on average, 55.71% (312 of the plants produced) of the total number of rootstocks grafted, after 1 vegetation.

Throughout the examination period, more first class walnut plants were produced by the in situ method (321) when compared with those produced by the indoor grafting method (299) (Table 4).

However, the applied technology for the production of the planting material for all of the varieties/selections examined did not show a significant influence on the success rate of the first class walnut plants. A statistically significant influence of the applied production technology was seen only for the G 251 variety, for which there were a lot more plants produced by the in situ method (80) than by the indoor grafting method (63). On the other hand, the influence of a variety, depending on the technology applied, on average, for the examination period, was notable. A statistically more significant success rate for the first class walnut plants was found with the Rasna selection (102 with the in situ method and 90 with the indoor grafting method), as it was for the Šampion variety (89 with the in situ method and 74 with the indoor grafting method), and at the same time, the G 251 variety reached a significantly and constantly high number of first class walnut plants (80). A significantly smaller number of first class walnut plants were produced with the Jupiter variety by both the in situ and indoor grafting methods (56 and 57, respectively) in comparison with all of the other varieties and grafting methods, except for the G 251 variety grafted by the indoor grafting method.

The advantages of the in situ method of walnut planting material production in comparison with the indoor method are as follows:

The production of good quality plants is done in 1 vegetation period, the grafting success of the planting material is better, and the many operations that are typically applied in the traditional method that decrease the success rate of indoor walnut grafting are excluded from the process. Avoiding all of these, makes in situ technology a much easier, faster, and cheaper method of walnut plant production in comparison with the indoor method, which has thus far been applied.

Table 3. Production of first class walnut plants by in situ grafting (2015–2016).

Variety/selection	No. of grafted rootstocks	No. of plants produced after the first production year	
		Compared to the grafted plants (%)	First class plants (%)
Rasna	140	72.86	72.86 a
Šampion	140	57.14	52.86 b
G 251	140	57.86	57.14 b
Jupiter	140	40.71	40.00 b
Total/average	560	57.14	55.71

* Values in the same column with different letters were significantly different according to the Duncan test ($P < 0.01$).

Table 4. Influence of the applied technology on the production of the planting material of the varieties/selections examined on the production of first class walnut plants.

Variety/selection	No. of first class walnut plants depending on the applied technology	
	Indoor grafting (after the second year)	In situ grafting (after the first year)
Rasna	90 a	102 a
Šampion	89 a	74 a
G 251	63 b	80 a
Jupiter	57 b	56 b
Total	299 A	312 A

* Values in the same column with different letters were significantly different according to the Duncan test ($P < 0.01$).

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