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RESEARCH ON THE IMPROVEMENT OF THE PRODUCTION TECHNOLOGY OF WALNUT GRAFTED PLANTING MATERIAL

ABSTRACT

The walnut, considered among the oldest fruit species found worldwide, enjoys the privilege of being among the healthiest fruits for human consumption.

Comparing with the other tree species, walnut propagation by grafting is more difficult, the yield of trees obtained being somewhat reduced. Its morphological and physiological peculiarities, closely related to climatic factors, make the specific processes after grafting (callus formation, welding and vascularization) more difficult.

In order to obtain grafted walnut planting material, numerous researches have been carried out different work regarding the grafting technique and conditions. (Constantinescu et al., 1937, 1964; Cociu et al., 1960, 1972, 2006; Achim et al., 1998, 2001, 2007, 2016; Corneanu 1997, 2018; Comănici et al., 2005; Țurcanu et al. , 2004, Godeanu et al., 2004, Avanzato et al., 1997, 1998, 2001, Gandev et al., 2005, 2007, 2014, Karadeniz et al., 2003, 2004, 2011; Pinghai 1993; Rezzae et al., 2011, 2008; Vahdati, 2014, 2016; Mir et al., 2020).

Results of these researches, except bench grafting and *in vitro* grafting, failed to be confirmed year after year. In the case of walnut grafting in the open field, the results obtained are most often influenced by the climatic conditions of the respective year and the area where study was performed.

The doctoral thesis with the title ***Research on the improvement of the production technology of walnut grafted planting material,*** wants to bring an innovative contribution to increase the amount of grafted planting material, through the efficient use of biological material (varieties and rootstocks), grafting methods and periods and the conditions of growth and development of grafted trees.

During the research, experiences were organized in a protected space (polyethylene tunnel) as well as in the open field, which focused on the main technological aspects of the production of walnut grafted planting material.

The doctoral thesis includes 196 pages, 28 tables, 75, figures and 3 appendices, being structured in two parts and comprising seven chapters to which the bibliography is added.

Part I represents, in its four chapters, the documentary study from specialized literature that highlights the importance of walnut culture, the current research and production technology of grafted walnut.

Chapter I briefly highlights the importance of walnut culture and its fruits with high nutritional value, presenting at the same time, the evolution of walnut plantated areas and fruit production worldwide.

Chapter II summarizes research activities to date on grafted walnut production, highlighting existing grafting systems and methods.

Chapter III presents the biological bases of the production of walnut trees in a structured way, from production on own roots to obtaining *in vitro* material.

Chapter IV describes the technological sequences of planting material production, most frequently found in specialized fruit nurseries.

Part II highlights the results of the research activity, where data are presented, which concerned the study of the particularities of the growth of rootstock seedlings in the field and protected (polyethylene tunnel) area, results obtained regarding the production of grafted planting material under bench grafting followed by forcing conditions, open field and protected spaces (polyethylene tunnel).

Chapter V presents the study of the conditions offered by the natural setting of the farm, where the research was carried out. Based on the data analyzed regarding the climatic factors in the area there were found good conditions for the establishment of walnut orchards, and for the grafting period (April-May, August), it can be observed that there are not summed up the best conditions for grafting in the open field. The average temperatures for April and May were 11.9-17.2°C, and during 2017-2019, the average temperature of August was 22.6°C. As for the atmospheric humidity, it oscillated between 66-68% during April - May and 63% for August.

Chapter VI describes the purpose and objectives of the research, presenting the way of organizing the experience in close connection with the observations and determinations there were performed.

The biological material used is represented by three varieties 'Anica', 'Velnița', and 'Miroslava', obtained at S.C.D.P. Iași; 'Grădinar' variety, obtained at U.S.V. Iasi, and four local selections 'Săbăoani', 'Șorogari', 'Belcești' and 'Bălțati'.

The experiments concerning the grafting of all varieties and selections mentioned above, were performed in three different ways, respectively: bench grafting followed by forcing the material in protected spaces; walnut grafting in protected spaces (polyethylene tunnel) and walnut grafting in the open field.

In experiment I, there were made observations and determinations regarding walnut propagation technology, segments using bench grafting and forcing the material after grafting.

Experience II, regarding the production of walnut rootstocks, was of a bifactorial type, the experimental factors being the method of culture, with two graduations: in open field and polyethylene tunnel. The preparation method of walnuts for the establishment of field I, had also two graduations; V1 – *stratified* and respectively V2 -*unstratified*.

Experiment III, concerning walnut grafting in protected spaces and open field, was a trifactorial type experiment (2x2x3), the variants being placed according to the method of subdivided plots. The experimental factors and their rankings were as follows:

Cultivation mode (A): *a1= in open field*; *a2= in protected spaces* (solar).

Grafting method (B): *b1 = chip budding*; *b2 = in patch budding*.

Grafting period (C): *c1 = in August* (sleeping bud); *c2 = in April* (growing bud); *c3 = in May* (growing bud).

Chapter VII, in its six subchapters, summarizes the own results obtained for each stage of the technological processes.

In ***subsection 7.1.*** are presented the results obtained regarding the production of walnut rootstock seedlings in field conditions and protected areas (polyethylene tunnel). By using walnut fruits stratified for 90 days and sowing them in spring (March) when establishing field

I, in the polyethylene tunnel, germination percentage varied between 62.10 - 71.40 %. In the case of using unstratified fruits, sown in November, the germination percentage ranged between 57.49 - 64.30 %.

In field conditions, by using stratified fruits for 90 days and sowing them in spring (April), was obtained a germination percentage that varied between 55.63-65.62% and in the case of using non-stratified ones, sown in November, germination percentage oscillated between 51.97 – 57.49 %.

The determinations made, during the three years of study, on the rootstock seedlings obtained in the polyethylene tunnel, revealed an average height of 75 cm and 1.4 cm thickness, 10 cm from the soil surface.

Under open field conditions, rootstock seedlings recorded an average growth of 56 cm in height and 1.1 cm in diameter in the grafting area.

Subchapter 7.2. includes a study on the anatomical-morphological changes that take place in trees grafting area and the physiological and biochemical peculiarities of scion growth and development.

The anatomical-morphological changes that take place primarly at the grafting point level and ultimately lead to successful grafting, occur in three distinct stages: *callus formation, welding* and *vascularization*.

Microscopic observations on the cross-sections taken 15 and 25 days after budding, in the grafting area, highlighted both the presence of callus and undifferentiated parenchymal tissues, as well as the initiation of revascularization between scion and rootstock, in all walnut studied varieties, using the chip budding method.

The main leaf pigments, chlorophyll and carotenoid content, provide valuable information about the physiological status of plants.

In the four walnut grafted varieties, the content in chlorophyll pigments oscillated between 3,30 mg/g d.s. 'Grădinar' variety and 3,01 mg/g d.s. at 'Velnița' variety.

The chlorophyll a/chlorophyll b ratio, in studied varieties, fell within the theoretical limits, being between 2.37 mg/g d.s. in 'Miroslava' variety and 2,85 mg/g d.s. at 'Grădinar' variety.

Following the study carried out, the ratio of chlorophyll pigments/carotenoids recorded values between 2,63 mg/g d.s. in 'Grădinar' variety and 3,49 mg/g d.s. in 'Velnița' variety. These results highlight a normal development of the four walnut varieties grafted using the chip budding method.

In varieties with a low percentage of grafting ('Miroslava' and 'Velnița') an increase in peroxidase activity was highlighted, while in varieties with a percentage of over 50% ('Grădinar') the peroxidase activity was much lower.

In ***subchapter 7.3.*** are presented the results obtained when using bench grafting followed by forcing the material after grafting.

The results obtained after using perfected copulation grafting method during the vegetative rest period, highlight the percentage of 65.3 % success (average over the 3 years of study).

Subchapter 7.4. includes a comparative study, regarding walnut grafting in protected spaces (polyethylene tunnel) and in field conditions.

Analyzing the data obtained, regarding grafting during the entire period of study 2017-2019, it can be seen that the best results were recorded in the case of chip budding grafting, 51.4 %, with a significant difference compared to patch budding grafting in which an average of 33 % was obtained.

In open field conditions, in the case of grafting performed in April, the best results were obtained with the chip budding grafting method, respectively 13.33 % success grafting and patch budding grafting of only 10% success.

In the case of grafting performed in August, in protected (polyethylene tunnel) conditions, during the period of study 2017-2019, using the patch budding grafting method, the grafting success was 20% and respectively 19.66% when chip budding grafting method was used, without significant differences between the two variants.

In **open field conditions**, the best results (16%) were obtained with the budding patch grafting method. When chip budding grafting method was used, grafting success percentage was 15.66%, without significant differences between the two variants.

Analyzing the results obtained, regarding grafting success in all studied experimental variants, variant a2/b1/c2 (grafting in protected area - polyethylene tunnel / chip budding / growing bud - April) stands out, with a value of **51, 4 %,** with a significant difference compared to the other experimental variants.

Following the determinations made, it was observed that the two grafting methods presented a similar ***degree of callus formation*** depending on the analyzed grafting period. Reported from the point of view of the grafting period, the highest callus formation degree values (3.6-3.7) were obtained by grafting in protected space both in August and April.

In ***subsection 7.4.2.*** is analyzed the influence of the grafting method and the method of preparing the graft shoots, on the attachment to walnut grafting. Harvesting the graft shoots, for the dormant bud graft, will be done on the day or the day before grafting. The concentration of phenolic compounds in grafted shoots and rootstocks at the time of grafting is of particular importance in terms of grafting, because a high content of these compounds endangers grafting.

For the grafts performed in August, grafted shoots with vegetative buds from the current year were used, using the three experimental variants, for each grafting method used (chip-budding and patch budding).

In the case of grafting performed in protected spaces (polyethylene tunnel), the grafting rate recorded the highest values, in the V2 variant in which the grafted shoots were defoliated approximately 21 days before the date of grafting, keeping the leaves only at their top, 27.6% (grafting in chip budding) and respectively 25.1% (grafting in patch budding). Followed V3 variant, treated with vitamin C in concentration of 3 g/l, with grafting success percentages of 22.02 % (chip budding grafting) and respectively 20.7 % (patch budding grafting).

In case of grafting performed in the open field, the grafting success rate recorded the highest values in V2 variant, in which the grafted shoots were defoliated approximately 21 days before the grafting date, keeping the leaves only at their top, 18,4 % (chip budding grafting) and respectively 18,6 % (patch budding grafting).

Subchapter 7.4.3., presents aspects regarding the particularities of growth in field II of the nursery of the studied varieties and selections.

The recorded data on shoot growth dynamic show different values for each variety and selection, but which were within relatively close limits for both grafting methods.

In protected spaces (polyethylene tunnel), the most vigorous growths were recorded in walnut varieties and selections grafted in August in case of both grafting methods (a2/ b1/c1, a2/ b2/c1).

Among the varieties with the most intensive growth of the graft shoot, as in the field, 'Anica' variety stood out with a growth of the graft shoot around 200 cm, while at the opposite pole was the selection 'Șorogari' with 171 cm.

Graftings in growing bud from April recorded growths of the graft shoots between 184 cm at 'Grădinar' variety and 164 cm at 'Șorogari' selection.

The ratio between the diameter of the rootstock and the scion for the chip budding grafting method varied between 0.75 for 'Bălțati' selection and 0.95 for 'Grădinar' variety. For patch budding grafting technique, the ratios ranged between 0.78 for 'Anica' variety and 1.14 for 'Șorogari' selection.

Thus, in the case of grafting performed in open field in August, using chip-budding method, the growth of scion shoot oscillated between 155.4 cm in the case of 'Anica' variety and 124.5 cm in 'Șorogari' selection. 'Miroslava' variety, registered highest growth value, 141.7 cm, in case of patch budding grafting method.

For the field grafts performed in April, the degree of scion shoot growth was between 126.5 cm for 'Grădinar' variety, in the case of chip budding grafting, and 101 cm for 'Bălțati' selection, using the patch budding grafting method.

Subchapter 7.5. presents the analysis of the quality of the planting material obtained by walnut grafting.

The analysis of the root system is a quality parameter of the planting material, which is determined when the trees are removed from the second field of the nursery. The varieties and selections used presented a strong root system, most of the time pivoting with an average number of ramifications (5-6), uniformly arranged around the pivot exceeding 40 cm in length.

The studies carried out on the grafted material in the polyethylene tunnel showed percentages between 83.7% and 85.5% STAS grafted trees, in the case of grafting in patch budding and 92.2-95.4% using chip budding method.

In ***subsection 7.6.*** the economic efficiency of the production of walnut planting material is analyzed.

The economic efficiency in the production of walnut grafted planting material is closely related to the climatic conditions during the grafting period, the grafting percentage and the subsequent yield of STAS grafted trees.

The highest rate of profit was obtained in the production of planting material grafted in the polyethylene tunnel, respectively 188.02%, followed by bench grafting with 170.5%.

The technology of obtaining grafted walnut in the open field has a profitability rate of 158.7%.