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Theses of Doctoral (PhD) Dissertation

Development of high biological value grape propagating material based on environmentally friendly technology

Péter Szabó

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The doctoral school

name: Festetics Doctoral School

study field: Crop and Horticultural Studies

head of the doctoral school:

Dr. Anda Angéla D.Sc.

university professor, doctor of the Hungarian Academy of Sciences

Hungarian University of Agriculture and Life Sciences, Institution of horticultural sciences

Doctoral advisors: Dr. Kocsis László D.Sc.

university professor, doctor of the Hungarian Academy of Sciences

Dr. Pupos Tibor C.Sc.

university professor

The candidate fulfilled all the conditions prescribed in the Doctoral Regulations of the Hungarian University of Agricultural and Life Sciences, the remarks and suggestions made in the workplace debate of the dissertation were taken into account during the revision of the dissertation, therefore the dissertation can be submitted for defense.

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approval of the head of the doctoral school	approval of the doctoral advisor	approval of the doctoral advisor

1. Antecedents and objectives of the work

The characteristics and genetic values of the variety are embodied in the propagating material, which determines the condition and productivity of the vineyard, the quality of the final products, and ensures the competitiveness and profitability of viticulture for decades. High-quality, pathogen-free propagating material that ensures long-term competitiveness can only be produced with the active participation of all actors in the sector. The viticulture sector, more precisely the grape propagating material sector, is facing numerous challenges nowadays. There are also a number of problems to be solved with regard to genetic bases, cultivation technology and plant health aspects. Grape propagating material production is a dynamically developing, innovative sector worldwide. Climate change, the demands of producers and consumer and societal expectations pose new challenges for all actors, from the production of planting material to the marketing of wine. The survival and further development of the sub-sector can only be guaranteed by continuous innovation, preserving the existing values, adapting to the changing ecological and economic conditions (Molnár, 2019).

Based on the above, we aim to examine:

Our research goal was to develop the incorporation of new elements in the field of production of grape propagating material. We wanted to reduce the energy use of the technology, the use of oil-based agents, the infestation of pathogens, and other changes in the cultivation technology to produce the healthiest grafts possible in an environmentally friendly way.

Based on our previous results, we performed comparative studies on the three semi-forcing used in practice (sawdust, perlite, water). Our goal is to develop a new, innovative grape propagating material production technology (indoors (greenhouse) and free-root grape graft production with soilless technology) that is sustainable, environmentally friendly, and the materials used are recyclable. It was also an important goal for the new technology to be both water and nutrient efficient, while at the same time achieving an excellent origin and quality. We compared the conventional (outdoor and baked schooling) training technology with the technology we developed, which can be considered innovative, based on physical parameters and indicators that significantly determine the value of its contents (carbohydrate content of canes, leaf analysis). The results of the various studies were evaluated by statistical methods

2. Material and methodology

2.1. Presentation of the used natural elements

The main grape varieties producing white and red wine in Hungary and the rootstocks popular in the domestic and international production of propagating material were included in the study. A total of about 4059 plant data were processed between 2016 and 2019. All the plant materials used came from the same production site, the Georgikon Campus of the Hungarian University of Agriculture and Life Sciences (hereinafter: MATE Georgikon Campus) (legal predecessor: Georgikon Faculty of the University of Pannonia) in Cserszegtomaj (Table 5). In the course of our research we created the most important (Teleki 5C, Teleki-Kober 5BB) and varieties of Georgikon 28 bred on Georgikon from the main grape varieties in Hungary (Italian Riesling, Cserszegi fűszeres and Kékfrankos, Cabernet Sauvignon, Merlot).

2.2. Methodology for the preparation of propagating material

The technological process of carrying out the experiment was similar in each year. Here are the steps to do this: from picking to the planting into the nursery process. In the first step, in the second and third weeks of February each year, the canes of the grape rootstocks were harvested, cleaned, cut to size, bundled and graded according to thickness, which were placed in a cold store at a temperature of 1-5 °C until inoculation. It was protected from drying by covering with foil. The buds of the rootstocks were removed (blinded) and then planted before inoculation, followed by soaking. The scion varieties were also cleaned, cut to size, graded, and then bagged, and also stored until inoculation in the same manner as the rootstocks. Rootstock to grafting, the rootstock varieties were soaked for 5 days, while the scion buds were soaked only 2-3 hours before grafting according to the recommendations of the literature and the method used in practice.

2.3. The methodology of forcing

The rootstock and scion components used in the research were typically grafted with an Omega-type grafting machine in mid-April. For both technologies, the finished grafts were dipped in “Proagriwax G-Mediterranean” grafting wax. The basal end of the rootstocks was not treated with a rooting stimulant. After grafting, the propagating material was placed in plastic and wooden crates for semi-forcing. Perlite and sawdust was used to force the grafts. The new technological element was forcing in water. In this case, after grafting, the finished grafts were placed in plastic crates filled with water (up to about 2 cm water cover), but in this case no layering material was used. No precursor medium was used for control plants.

The grafts were placed in the forcing room in the second week of April each year. Forcing took place at the MATE Georgikon Campus Viticulture and Oenology Experimental Plant in Cserszegtomaj. During the first 5 days of the shoots we tried to keep the grafts unchanged at 28-32 ° C and constant humidity, then the room temperature was continuously reduced in accordance with the practical and literature recommendations. The hardened grafted grafts were hardened to accustom them to the outdoor environment.

In our study, we evaluated four different parameters on pre-propagated grafts:

- plantar callus development of the graft
- number of roots appearing at the basal end of the root canal (pcs),
- and unfolding the bud of the noble pin
- the quality of the callus of the graft.

2.4. The methodology of the conventional nursery technologies

After semi-forcing and training, the plants were planted into the nursery. The conventional and the newly developed technology are separated here. The schooling is the planting of forced grape grafts, which in the case of conventional technology takes place in the open field, in a nursery and typically in an open furrow, while in the case of innovative technology we used greenhouse, growing equipment and soilless technology.

The training was carried out outdoors in the manner already known. About 14 grafts were placed per meter. During the growing season, we also carried out the necessary green work, truncation, hunting (removal of shoots of the rootstock) and planned plant protection. The first spraying was carried out at the developmental stage of two to three leaves, then the plants were treated weekly (downy mildew, powdery mildew) until mid-July, from the second half of July to the end of August approx. sprayed every 10 days / fortnight. Irrigation was typically performed with a drip device, with nutrient replenishment (nutrient solution) in one operation. Rooted grafts and cuttings were collected from mid-October using a grafting plow.

2.5. The methodology of the carbohydrate analysis

The preparation of each sample for testing was preceded by the determination of the physical parameters of the grafts: the diameter of the cane, including the proportions of the tree-gut, and the length of the internode were recorded. The samples were then dried at 70 °C to determine their dry matter content. Each graft was separated into three fractions: the inner spongy, the woody, and the outer spleen. In the further studies, the intermediate woody part was used. The samples were ground to a fineness until the appropriate particle size for chemical analysis was reached. For the determination of the dry matter content, dry the test sample at a given temperature (70ºC) and determine the loss in mass. The levels of starch and high molecular weight starch degradation products were determined by polarimetric method. The essence of determining the cellulose content is to remove the lignin and hemicellulose extracts and then measure the weight of the residue. For sugar determination, the grape grafts were first dried, the fractions were separated, and after pulverization and homogenization, they were used to determine the sugar composition. To determine the sugar composition, a calibration series was prepared using external standards and injected into the HPLC apparatus. The assay was performed on an Agilent 1100 high performance liquid chromatograph (HPLC) and an Agilent 1200 refractive index detector (RID).

2.6. The methodology of the analysis of leaves

Due to the special nature of the experiment, the samples on which the leaf analysis study was based were taken in the last week of October each year after the experiments were broken down, as the nature of our research did not allow us to take samples before taking the experiment (destructive study). A sample consisted of about 100-150 plant organs (leaves).

2.7. Statisztikai elemzések módszertana The methodology of statistical analysis

All analyzes were performed at a significance level of 5% (95% confidence level). During our work we used ANOVA and Levene test, Éta and Cramer indicators to process the obtained results.

3. Results

3.1. Pre-propagation

Based on our results, it can be stated that we achieved the best forcing results in the sawdust forcing medium. In perlite medium, the appearance of the plantar callus, the bud sprouting rate and rooting gave the best results.

In our opinion, a uniform temperature and humidity can be ensured for the grafts in the perlite and sawdust medium. The plant is more sensitive to sudden temperature changes in aqueous environment. The grafts propelled in perlite and sawdust do not dry out and the temperature fluctuations are smaller in these media. From the results of our research, we came to the conclusion that the inoculation of scion vines grafted on different rootstocks is greatly influenced by the pruning technologies used in the production of grape grafts.

3.2. Effect of carbohydrate content on the inoculation of grapes

In our work, we showed a significant relationship between the quality of the callus and the starch content of the grape propagating material. Thus, we found that the higher the level of starch, the more it develops, forming the wound-welding tissue along the cane-cambium, that is the so called callus. Our results are in line with the findings of Pánczél and Eifert, 1961, that the development of new organs, and thus the expected viability of the new individual, is related to the amount of accumulated energy source (carbohydrate). We also conclude that the amount of carbohydrates, and within that, starch, is perhaps less important, and their mobilizability may be more decisive.

3.3. Comparative analysis of the physical parameters of free-rooted grafts grown with soilless technology and grape grafted with conventional technology

Based on the results, we found that the percentage of origin was better than in the field grafts in terms of sprouting the buds of the noble pin. In terms of cane diameter, we also recorded larger data for field plants. All this is probably due to the fact that the grafts grown in the open field had a better supply of nutrients. In terms of root number, the soilless technology proved to be better: apparently a much larger number of roots were grown in the vines, although it should be added that in terms of root thickness, the field grafts proved to be better. We obtained almost the same average results in root development: in this parameter both the field and the new technology tested well.

Overall, it can be concluded that the new technology, which is considered to be important in terms of important physical parameters in the graft production technology, competes with the conventional technology. In terms of cane ripening and root thickening, further technological developments may be desirable. As the most important feature of soil-free propagating material production is that the growing medium is not soil, the basic functions that soil provides to the plant must be ensured in a different way. We need to find an answer to this problem.

3.4. Examination of carbohydrate content of grape grafts indoors, with soilless technology and trained with conventional technology

According to our results, only in the case of glucose there was a significant relationship based on the differences in both means and standard deviations. In the case of fructose, only the standard deviation of the measured values differed significantly from one medium to another, but the average did not.

3.5. Leaf analysis studies in greenhouse conditions on vine grafts grown with soilless technology

The aim of our studies from 2016 to 2019 was to develop a nutrient solution technology for soilless graft production technology. Based on our experience, we have tried to continuously adjust the nutrient supply of the plants. In many cases, the different nutrient levels and nutrient ratios also depend on the nature of the nutrient solution and its usage. Based on the results, it can be stated that soil-free cultivation can be just as effective as field cultivation, and an adequate supply of nutrients can be ensured for our plants in this case as well. Based on all this, we can conclude that the nutrient supply technology of soilless education does not lag behind the level of field-free nursery technology. Thus, in four years, the nutrient solution technology for the production of soilless grape grafts has been developed.

3.6. Investigation of the origin of grape grafts grown indoors with soilless technology after planting and the effect of carbohydrate content on the percentage of origin after planting the grafts

Based on our results, we can state that the soilless technology does not lag behind the conventional technology in terms of the percentage of origin after the planting of the vine. Although no major difference was observed, the field mean was 0.8, the soilless mean (0.82). indicator, all of which shows a weak relationship. Of the other variables to be explained, only the ANOVA and Levene tests became significant only in the case of starch and sucrose, ie only then could the Eta index be interpreted, all of which show a weak relationship. The medium has a weak effect on the starch and sucrose content of the grape graft. In the case of fructose, only the average of the measured values differed significantly from one medium to another, but the standard deviation did not. Based on the value of Eta, this indicates only a very weak relationship. The standard deviation of the measured values differs significantly for the following factors, but their average is not: dry material, moisture, cellulose, total carbohydrate. In the case of the first three, the relationship can be called weak according to the Eta, but in the case of the latter (carbohydrate) it is strong.

3.7. Technological description - Production of free-rooted grape propagating material in confined spaces without soil technology

The technology of the developed new graft production and training method up to the nursery phase is the same as the conventional technology. It is recommended to force in sawdust or perlite media if a forcing room with a fully automated air conditional is not available. It is almost clear that in the case of soilless technology, the planting does not take place in the open field, so there is no need for land or the use of nursery rotation, ie soil preparation, during planting into the nursery. In addition to the field planting, in our experiment, in the case of the technology that can be called innovative, the pre-sprouted, free-rooted grape grafts were placed in a greenhouse, growing equipment and without soil technology.

Prior to planting into the nursery, we prepared the grafts to be planted and treated them with grafting wax. The plants to be planted were placed by hand, but not in an individual tray (as in the case of container grafting), but in a common nursery, so we are talking about free-root grafting. Approximately 625 grafts per 1m^2. (Figure 1).

Figure 1 – The experiment (grafts in 1m^2)

In practice, the (plastic) container used during forcing was used as a training device. Plastic inserts for isolation of plants, separation between stems and plant fixation can also be used. As for the growing and root fixation medium, we chose perlite. In practice, the CTFI REG (plastic) container used during pre-propagation was used as a growing equipment. The growing equipment is designed to be an open system. When designing the open system, we made sure to deliver only as much nutrient solution to the plants as is absolutely necessary in order not to pollute the environment. A rescue tray was designed to drain the nutrient solution, which can be slid under the box container. The operation of closed systems requires greater professional and technical training. Nutrient supply and irrigation were achieved simultaneously with programmed nutrient solution irrigation using macro- and microelements developed for the vine. When watering the grafts, special attention was paid to ensure that the root fixation medium did not dry out below 60-70% of the field water capacity. Adequate nutrient supply was provided from 2016 to 2018 with fertilizers from the YARA product family (Kristalon blue, Kristalon yellow) in a dose of 1.5 kg / 1000 liters. The “Kristalon Blue” product is recommended for the vegetative phase of plants, which was used as a continuous nutrient solution. In 2019, the experiment was performed at Tapolca, Mogyorósi Szőlőoltvány Kft, under large-scale conditions. The provision of a suitable humidity is ensured by a suitable humidifier. For plant protection, only contact preparations were used if necessary. In 2016, we did not use pesticides, while from 2017 we used conventional technology, similar to the open-air graft school. Regarding cultivation work, truncation (cutting back shoots to about 40-50 cm) was performed during the growing season. Occasionally, hunting was also needed (removal of shoots of the rootstock). Pick-up was done by hand, in which case no other tools, equipment or machines were used.

Conclusions, suggestions

In today's world that is difficult to sustain - and increasingly threatened by climate change - the importance of soilless grape graft production can be expressed in terms of **sustainability**, **environmental awareness** (reducing environmental impact) and **recyclability**. Due to the optimal structure, it is easier to ensure an **optimal water and nutrient supply for the grafts**.

Table 1- The SWOT analysis of the innovative, newly developed soilless grape grafting technology

Strenghts	**Weaknesses**
+ The use of precision tools and equipment can reduce the possibility of human error. + The technology does not require organic manure, which is becoming increasingly difficult to obtain. + Much less plant protection is required. + We do not spread soil pathogens or pests. + With this technology, grafting is easier and cheaper. + No power equipment requirements. + We can provide adjustable conditions. + Not affected by extreme weather. + Improves crop safety and predictability, competitiveness. + Does not require significant land.	– Specific investment costs are higher than with conventional technology. – In case of operational application, an advanced, complex technical system must be used. – The tools and the equipment requires higher service expenses. – Requires great technological discipline. – It requires a high level of knowledge, accuracy and special expertise. – A well-functioning service network is needed. – It assumes a reliable energy supply. – Disposal of the used root fixative media is expensive and sometimes difficult to solve.
Possibilities	**Dangers**
+ Harnessing the need for sustainability due to climate change. + Reduction of control costs against pests and pathogens. + Reduction of environmental impact. + Take advantage of more green program support opportunities.	- Increase in replacement costs due to slightly lower quality in vine maturation. - The root will be less thick than with conventional technology, so storage technology can be critical.

In summary, it can be stated that the production value of the open-air graft nursery is high, so even in the case of a minor infection, the producer may suffer greater loss. In the case of plants grown indoors with soilless technology, the problems caused by the weather and pests are much more eliminated. So: the production risk is much lower or even negligible.

Based on the results, it can also be stated that soilless cultivation can be just as effective as field cultivation, and an adequate supply of nutrients can be ensured for our plants in this case as well. Based on all this, we can conclude that the nutrient supply technology of soilless grafting has been developed, but of course further clarifications can be made. The great advantage of the system is that it can be used anywhere on Earth in the production of soilless grape grafts in a growing plant, as there are no influencing factors (soil, different climate, etc.) that would affect it.

In our point of view, free-root graft production in the greenhouse could play an increasingly important role in the future, reducing the losses caused by extreme weather events and producing our plants in a much safer, more environmentally friendly way and in a much smaller area. It is also important to emphasize the fact that labor shortages in the sector and the increase in all costs of graft production necessitate the development of labor- and cost-saving technologies.

Soilless free-root grape graft production is an innovative technology developed for practical implementation. Our research results are encouraging. Its applicability will be decided by the graft growers and how they adapt the technology to their own cultivation options and to the receiving markets.

New scientific results

1. We proved a significant relationship between the quality of the pre-propagation medium and the budding of the grape graft.
2. We have proved that the rooting and post-planting origin of free-rooted grape grafts from soil-free cultivation may exceed the similar values of grafts made from the same raw material from the open-air graft school.
3. We have determined the complete technological procedure for the optimal leaf analytical parameters and nutrient replenishment of closed-system, free-root soil-free grape graft production.
4. We showed a significant relationship between callus development and the starch content of grape propagating material. The higher the level of starch, the greater the occurrence of callus tissue along the cane-camium.
5. We have developed the method and equipment for the production of closed-system, free-rooted soil-free grape grafts, the description of the technology.

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