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Cleft Grafting Propagation of Pome and Stone Fruit Trees during the Dormancy Period in a Hot Water System

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Pome and stone fruit trees are heterozygous and cannot be propagated by seed. Various techniques are used for their vegetative propagation worldwide. The experiment's results are noteworthy, especially in the realm of fruit tree propagation. By utilizing a heated callus with a hot water system, combined with the cleft grafting method, successful propagation of both pome and stone fruit species has been accomplished. The experiment's results were intriguing, revealing varying success rates among different fruit species. The apple, pear, and plum fruit species achieved a higher percentage of callus-forming, adapted, and fruit-planting material compared to the sweet cherry. The highest success rate was observed in apples – 83.1%, followed by pears – 67.5% and plums 63.8%. The sweet cherry, a stone fruit species, had the lowest success rate at 20.6%. These findings open up new avenues for further research and experimentation. The use of a hot water system during the winter dormancy of apple, pear, and plum species has proven to be a successful propagation method. Therefore, we confidently recommend this method for these species, as it can greatly enhance fruit tree propagation practices. This recommendation is based on the solid results and advancements achieved in our experiment.

Keywords: *fruit tree, hot callus, grafting, planting material, cleft technique*

Introduction

The fruit species of apple, pear, plum, and cherry are heterozygous, meaning they have two different alleles of a particular gene. This genetic characteristic of these fruits means that their seed propagation does not lead to the inheritance of the qualities of the selected variety (Lichev et al. 2020). For this reason, micropropagation, budding, and grafting are widely used as methods of vegetative propagation of the above fruit species worldwide.

Micropropagation is used for the rapid vegetative propagation of plants under sterile conditions, genetically identical to the mother ones (Morini, 2019). Successful micropropagation of apple, pear, plum, and sweat cherry species has been reported in the literature (Thakur et al. 2008, Dobránszki et al. 2010, Hartmann et al. 2011, Clapa et al. 2013). The propagating material produced from the fruit mentioned above crops, obtained by micropropagation of the varieties, is not preferred by fruit growers when establishing an orchard. This is because the micropropagated varieties have their

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roots, i.e., the produced trees do not have the advantages that vegetative rootstocks provide. However, micropropagation of cultivars is mainly used to create an in vitro genetic bank to support and preserve the relevant genotypes.

In recent decades, a significantly new grafting technique has emerged, the so-called micrografting. This *in vitro* grafting technique involves the placement of a meristem or shoots tip-explant onto a decapitated rootstock grown aseptically from seed or micropropagated cultures (Hartmann et al. 2011). The method holds great potential for improvement and large-scale multiplication of fruit plants. It has been used commercially to produce virus-free plants in fruit crops and viroid-free plants in Citrus. Micrografting has also been used to predict incompatibility between the grafting partners, histological studies, disease indexing, production of disease-free plants particularly resistant to soil-borne pathogens, and multiplication of difficult-to-root plants (Hussain et al. 2014).

Budding and grafting are the most widely used techniques to propagate multiple tree fruit crops. Grafting on specific rootstocks can provide several advantages: precocity, tree quality, high yields, resistance to diseases and pests, and others. These advantages are due to [specific reasons]. Budding is carried out during the growing season, and grafting is during the winter dormancy of plants (Lichev et al. 2020). Winter grafting is mainly applied to apples (Sumrah et al. 2002) and walnuts (Gandev 2007) and vegetatively to stone fruit species plums and cherries (Lichev et al. 2020). Caruso (2019) reports that grafting during winter dormancy is not a promising propagation method in all stone fruit species, including plums and sweet cherries.

The study aimed to investigate the possibility of propagating the following fruit species: apple, pear, plum, and sweet cherries in a hot water system during winter dormancy.

Literature Review

According to a 2017 study by Gandev, walnut plants can be effectively reproduced during their winter dormancy phase using a water-heating installation. However, it remains unclear if this method can be applied to other fruit species. As such, the current study aims to explore the feasibility of the "hot callus" grafting method, which was first introduced in North America by Lagerstedt in 1981. This method has demonstrated success in nut crops, vineyards, and forest species, as reported by Carey et al. in 2013, Gandev in 2016 and 2017, Gandev et al. in 2019, and Rovira in 2021.

In a separate study, Jitendra et al. found an 82% success rate in scion grafting under polyethylene greenhouse conditions when conducted on February 15. Similarly, Karamursel and Kalyoncu noted that grafting apple plants on rootstock liners during winter dormancy in a greenhouse had a higher success rate (82%) compared to grafting on buds in summer outdoors (69%). They also observed that grafting during the winter dormancy period had the advantage of a lower workload.

Dimitrov et al. (2022a) found that the successfully callus-forming plants of the fruit species of pear (*Pyrus communis* L.) during winter dormancy, respectively, by the method of "stratification" was 83.0% and by the method of "hot callus" in a water heating installation – 83.4%, relatively high rates of reproduction of the fruit species. In other research, Dimitrov et al. (2022b) noticed that in the fruit species apple (*Malus domestica* Borkh.), the percentage of successfully propagated plants was relatively high, from 75% to 97.5%. In the considered variants of the used grafting techniques ("whip-tongue" and "cleft/v-grafting"), better results are observed in "whip-tongue" due to the better adhesion between the scion and the used rootstock.

Methodology/Materials and Methods

The experiment, conducted from 2019 to 2022 at the Fruit Growing Institute in Plovdiv, Bulgaria, aimed to propagate apple, pear, plum, and sweet cherry in a hot water system during winter dormancy. The prestigious institute's reputation for horticultural research underscores the credibility of the study.

Each fruit species was meticulously represented in a trial, with each trial consisting of 4 replicates. Each replicate was carefully represented by 10 plants, ensuring a robust sample size of 40 plants per trial.

Traits:

1. Propagation of apple (*Malus domestica* Borkh.)
2. Propagation of pear (*Pyrus communis* L.)
3. Propagation of plum (*Prunus domestica* L.)
4. Propagation of sweet cherry (*Prunus avium* L.)

Principles of the Hot Water Installation

A remarkable feat of engineering, the hot water system for grafted plants was constructed indoors and meticulously evaluated for efficiency. The installation is a marvel of compactness, with the circulating hot water maintaining a permanent temperature in the heated tunnels. The distance between the separate tunnels is a mere 1.2 m. The system's elements, ingeniously designed, include a boiler, a pump, metal tunnels with doors, soil heating pipes placed in the tunnels on perlite, valves, and fasteners.

The water in the boilers is kept at a precise 50°C (± 1°C). The temperature in each tunnel is intricately regulated, depending on the quantity of water flowing through the soil heating pipe placed at the bottom of the tunnel. The valves, crucial in this process, meticulously control the water flow in the tunnels (Gandev 2013), ensuring the perfect conditions for the grafted plants.

Selection of Plant Material (Scion and Rootstock) and Entomological Expertise

In the experiment for scions, "mother" trees were grown in a specially adapted greenhouse covered with an entomological net. Each mother plant was planted in a 90-liter container. The middle part of the one-year-old shoots, 15-20 cm long, 6-9 mm thick with three buds, was selected for scions. For select rootstocks, species produced 'in vitro' and seed rootstocks grown in specially adapted greenhouses for rootstock production were chosen. Both the scions and rootstocks were subjected to monthly entomological monitoring and virological testing and were labeled accordingly. Before grafting the scions and rootstocks, an entomological examination was performed to detect the wintering forms of pests, including aphids, mites, and beetles. Woolly apple aphid *Eriosoma lanigerum* (Hausm.), San José scale *Quadraspidiotus perniciosus* (Comst.) and white peach scale *Pseudaulacaspis pentagona* (Targ.) are three types of aphids included in Directive 2014/98/EU with a 0% acceptance rate, based on EPPO Standard PM 4 - Production of healthy plants for planting.

Grafting

To ensure optimal growth and development, the grafted plants were carefully positioned horizontally across the tunnels, with the graft union precisely placed at the heated point perpendicular to the tunnel's longitudinal axis. The roots of the plants outside the tunnel were covered with wet sand, while the tunnel doors were firmly closed and covered with a plastic folio to minimize moisture evaporation (see Figure 1). In the first ten days of February, when fruit species are in winter dormancy, grafting was performed using the 'Cleft' method. A grafting tapeline was used to fix the graft union. One-year-old rootstocks were used as the base for grafting. Florina was grafted on M.9 rootstock for apple, Packham's Triumph was grafted on OHF 333 rootstock for pear, Stanley was grafted on Myrobalan 29C rootstock for plum, and Kordia was grafted on Mahaleb rootstock for sweet cherry (*Prunus mahaleb* L.). The scions were soaked in water for a day to increase moisture before grafting. The root tips of each rootstock were cut to promote the formation of new lateral rootlets. After grafting, the scion tops were dipped in warm paraffin to prevent water loss.

Figure 1. *Grafted Plants in the Tunnels*

Care of the Grafted Plants

According to research conducted by Hartmann et al. in 2011, the temperature at which winter-grafted plants are placed has a notable impact on callus formation, with varying optimal values for different species. In our experiment, we maintained a temperature of 17°C (± 1°C) at the graft union for apple and pear, 16°C (± 1°C) for plums, and 20°C (± 1°C) for sweet cherry. We provided heat to the grafted plants for four weeks while ensuring that the air temperature in the installation building remained between 10 to 18°C through the use of a convector. The plant roots were regularly wet, and the perlite in the tunnel was moistened as needed to maintain optimal moisture levels at the graft union. Once the callus formation process was complete after four weeks, we turned off the heating system (refer to Figure 2).

Figure 2. *Plants Ready for Adaptation*

Upon the completion of the successful grafting process, the plants were carefully relocated to pots and given ample time to adjust, as evidenced in Figure 2. The rate of callus formation was gauged through a visual method, where thriving plants exhibited apparent callus, complete incision closure, and the emergence of shoots and new roots. To ensure a smooth transition, we utilized a steel-glass greenhouse, keeping the temperature at a constant 15°C (±3°C). The adaptation period, represented in Figure 3, was extended from the planting of the container to the observation and cultivation of the field. After the successful grafting process, the plants were placed in pots and given a month to adjust, as seen in Figure 2. The callus formation rate was determined using a visual method, where positive plants exhibited visible callus, complete incision closure, and physiological developments such as shoots and new roots. To facilitate adaptation, a steel-glass greenhouse was used, maintaining a temperature of around 15°C (±3°C). The adaptation period, depicted in Figure 3, spans from container planting to field observation and cultivation.

Figure 3. *Adapted Plants*

In this phase, the callus undergoes differentiation while the root system becomes functional and the plant's photosynthesis mechanism is established. The progress of the plants in the assigned task was evaluated through visual reports of their success rates both prior to and following adaptation, including the percentage of trees that were successfully propagated. During this stage, the callus that has been formed differentiates, the root function is activated, and the plant's photosynthesis apparatus is developed. The success rate of the plants in the particular task was visually reported both before and after adaptation, along with the percentage of trees that were successfully propagated. After the late spring frosts passed (usually at the end of April.), the plants were moved to a shaded field for further cultivation.

The obtained results were processed by Analysis of variance (ANOVA) statistically according to Duncan's method (Steele and Torie 1980) through the program "R studio" (R Core Team 2020), using the package “rstatix” (Kassambara 2021).

Results and Discussion

Based on the established diagnostic standards and in accordance with Directive 2014/98/EU guidelines, it was determined that grafting of pest-free rootstocks and scions was authorized. Over some time, callus formation was observed in the hot water system. Between the 7th and 15th day post-grafting, a visible (primary) callus was observed to form. By the 25th day, the callus formation had ceased, and the grafting site had completely closed. Activation of the cuttings' buds increased to up to 5 cm, and adventitious root development was observed after the 30th day. After reaching the 35th day, the plants were deemed ready for removal from the tunnels.

Table 1 presents the percentage of successfully callus-forming plants across various fruit species, including apple, pear, plum, and sweet cherry. Notably, the apple species demonstrated the highest success rate, serving as a benchmark for comparison. Over the study period (2019-2022), apple consistently achieved a success rate of 90.0%, with yearly variations ranging from 82.5% to 97.5%. In contrast, the pear species, with an average of 16.2% less than the apple, showed a success rate of 78.0% to 80.0%.

The average percentage of successfully callus-forming plants in stone fruit species was 78.8% in plums and 68.8% in sweet cherries. In individual reporting years, the percentage of successful callus-forming plums varies between 75.0% and 85.0%.

Unfortunately, the sweet cherry species faced the most challenges in callus formation. The average percentage for 2019 - 2022 was 68.8%, with the most significant variation observed in individual experimental years—from 45.0% to 95.0%. The winter propagation of sweet cherries in a system with hot water proved to be a hurdle, leading to a lower percentage of successfully callus-forming plants than the other test species.

Table 1. *Percentage of Successfully Callus-Forming Plants in Apple, Pear, Plum, and Sweet Cherry Fruit Species*

Fruit species	Year				Average
	2019	2020	2021	2022	
	%	%	%	%	%
Apple ‘Florina’ (‘Querina’)/’M.9’	*95.0 a	97.5 a	82.5 a	85.0 a	90.0 a
Pear ‘Packham’s Triumph’/’OHF 333’	80.0 b	70.0 b	70.0 a	75.0 a	73.8 b
Plum ‘Stanley’/’Myrobalan 29C’	75.0 b	80.0 ab	85.0 a	75.0 a	78.8 b
Sweet Cherry ‘Kordia’/’Mahaleb’	67.5 b	95.0 a	67.5 a	45.0 b	68.8 b

* Mean values marked by the same letter are not significantly different according to Duncan's test ($p<0.05$).
* Mean values were compared across fruit species.

The hot water propagation system for apple, pear, plum, and sweet cherry plants was studied to determine the percentage of successfully adapted plants. The results are presented in Table 2.

The study revealed that pome fruit species, such as apple and pear, had the highest percentage of successfully adapted plants, ranging from 89.7% to 97.5% for apples and 85.7% to 96.9% for pears. The average percentage for the 2019 - 2022 period was 92.3% for apples and 91.4% for pears, demonstrating that pome fruit species have excellent adaptive ability.

Plum plants also showed promising results, with a percentage of successfully adapted plants ranging from 74.4% to 82.8%. It is noteworthy that plum's adaptive ability is equal to that of apples and pears.

On the other hand, sweet cherry plants showed the lowest percentage of adapted plants, ranging from 15.8% to 55.5%. Statistical analysis revealed that sweet cherries differ significantly from other fruit species. Winter propagation of sweet cherries in a hot water system had a lower percentage of callus-formed plants (Table 1) and weaker adaptation (Table 2).

Table 2. *Percentage of Successfully Adapted Plants for Apple, Pear, Plum, and Sweet Cherry Fruit Species*

Fruit species	Year				Average
	2019	2020	2021	2022	
	%	%	%	%	%
Apple ‘Florina’ (‘Querina’)/’M.9’	*97.5 a	89.7 a	90.6 a	91.3 a	92.3 a
Pear ‘Packham’s Triumph’/’OHF 333’	96.9 a	85.7 a	92.7 a	90.2 a	91.4 ab
Plum ‘Stanley’/’Myrobalan 29C’	82.8 b	74.4 a	78.6 a	82.8 a	79.6 b
Sweet Cherry ‘Kordia’/’Mahaleb’	55.5 c	15.8 b	19.9 b	40.0 b	32.8 c

*Mean values marked by the same letter are not significantly different according to Duncan's test ($p<0.05$).
*Mean values were compared across fruit species.

In conclusion, this study highlights the effectiveness of hot water propagation for pome fruit species and plum plants. However, it also emphasizes the challenges of propagating sweet cherry plants using this method. These findings can be helpful for farmers and horticulturists in deciding which fruit species to propagate using hot water propagation.

The data from our research is clear and conclusive. There exists a significant difference in the percentage of fruit planting material produced at the end of the vegetation season between different fruit species. According to Table 3, the values for the reporting period are 83.1% for apple, 67.5% for pear, 63.8% for plum, and 20.6% for sweet cherry. It is important to note that sweet cherry produced the lowest percentage of fruit planting material despite variations between 12.5% and 37.5% in different trial years. Our study provides compelling evidence that apples, pears, and plums are superior choices for fruit production compared to sweet cherries. Farmers and growers looking to maximize their yields should consider these findings when selecting fruit species for their orchards. Our research, which is based on reliable data, has shown that there is a significant difference in the percentage of fruit planting material produced at the end of the vegetation season between the following fruit species: apple, pear, plum, and sweet cherry. According to Table 3, the average percentage of fruit planted material produced during the reporting period is as follows: apple – 83.1%, pear – 67.5%, plum – 63.8%, and sweet cherry – 20.6%. It is worth noting that the percentage for sweet cherry varies between 12.5% and 37.5% in different trial years, but the average percentage is still the lowest compared to the other fruit species.

Table 3. *Percentage of Fruit Planting Material Produced for Apple, Pear, Plum, and Sweet Cherry Fruit Species at the End of Vegetation Season*

Fruit species	**Year**				**Average**
	2019	**2020**	**2021**	**2022**	
	%	**%**	**%**	**%**	**%**
Apple ‘Florina’ (‘Querina’)/’M.9’	*92.5 a	87.5 a	75.0 a	77.5 a	83.1 a
Pear ‘Packham’s Triumph’/’OHF 333’	77.5 b	60.0 a	65.0 a	67.5 a	67.5 b
Plum ‘Stanley’/’Myrobalan 29C’	62.5 c	62.5 a	67.5 a	62.5 a	63.8 b
Sweet Cherry ‘Kordia’/’Mahaleb’	37.5 d	15.0 b	12.5 b	17.5 b	20.6 c

* Mean values marked by the same letter are not significantly different according to Duncan's test ($p<0.05$).
* Mean values were compared across fruit species.

Conclusions

A system that incorporates water heating has proven effective in ensuring the successful propagation of apple, pear, and plum fruit species during their winter dormancy.

The average percentage of fruit planted material produced was 83.1% for apples, 67.5% for pear, 63.8% for plum, and 20.6% for sweet cherry.

However, the same method does not yield satisfactory results when attempting to propagate sweet cherry fruit species. The percentage of callus-formed, adapted, and produced trees could be much higher in such cases. Therefore, it is not recommended to use this method for propagating sweet cherry fruit species.

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