PhD THESIS

The Influence of Cultivation Systems on the Physical and Biochemical Quality of Apples

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INTRODUCTION

In the current context of sustainable agriculture and the increasingly demanding market requirements for high-quality horticultural products, modern fruit growing is faced with the need to reassess traditional cultivation systems and adopt technological strategies that maximize both economic efficiency and the nutritional value of the fruits. The apple (*Malus domestica* Borkh.), one of the most widely cultivated fruit species worldwide, holds a strategic role in the horticultural sector due to its ecological adaptability, high nutritional value, and versatility in consumption.

In this context, dwarf rootstocks such as M9 and semi-dwarf rootstocks like MM106 are extensively used in intensive and semi-intensive systems, offering distinct characteristics regarding tree development, yield per unit area, and influence on the physical and biochemical parameters of the fruits. Recent studies emphasize that the interaction between cultivar, rootstock, and technology can significantly influence apple quality, affecting traits such as firmness, density, sugar content, organic acids, vitamin C, and antioxidant bioactive compounds.

STRUCTURE OF THE PHD THESIS

The doctoral thesis entitled "**The Influence of Cultivation Systems on the Physical and Chemical Quality of Apples**" is structured into 10 chapters, which include 41 figures and 34 tables. The thesis is divided into two main parts:

The first part is dedicated to the **Current State of Knowledge** in the field of the chosen topic. It is structured into 3 chapters and spans 36 pages, representing 21.66% of the total thesis.

Chapter 1: Importance, Origin, and Cultivation Area of the Apple highlight the economic and nutritional importance, the origin and geographical distribution, genetic and biological characteristics, as well as modern cultivation systems, with a focus on essential technological links such as pruning, fruit thinning, and fertilization to achieve consistent and high-quality yields.

Chapter 2: Aspects Regarding Rootstocks Used in Apple Cultivation describes the classification of rootstocks used in apple growing based on their vigor, emphasizing the characteristics and role of vigorous, semi-vigorous, and dwarf rootstocks in influencing tree development and cultivation systems.

The second part represents the Personal Contribution, which extends over 94 pages, encompassing 8 chapters and representing 69.62% of the thesis. This section describes the research conducted during the period 2018–2020, grouped into four individual studies as follow:

Study 1 – Influence of Cultivar and Rootstock on Morphological and Productive Traits of Apple Trees analyzes how different combinations of apple cultivars and rootstocks influence the morphological development and production performance of the trees. The evaluations targeted aspects such as tree vigor, crown dimensions, fruiting capacity, and yield quality and quantity.

Study 2 – Influence of Cultivar and Rootstock on the Physical Characteristics of the Fruits analyzes how different cultivar–rootstock combinations affect the physical traits of apples, such as size, weight, shape, firmness, and commercial appearance.

Study 3 – Influence of Cultivar and Rootstock on the Chemical Characteristics of Fruits investigates how various combinations of apple cultivars and rootstocks influence the chemical composition of the fruits, highlighting parameters such as soluble solids content, acidity, vitamin C, and other bioactive compounds.

Study 4 – Influence of Storage Conditions, Cultivar, and Rootstock on Fruit Quality analyzes how post-harvest factors, alongside cultivar–rootstock combinations, influence the maintenance of the fruits' physico-chemical qualities during storage. Aspects such as weight loss, firmness, and changes in sugar, acid, and bioactive compound contents (such as carotenoids and total phenols) are evaluated.

The doctoral thesis concludes with **Conclusions and Recommendations**, followed by a section on the **Originality and Innovative Contributions** of the research.

The Bibliography includes 389 references, comprising both domestic and international sources, as well as web-based materials.

HYPOTHESIS AND RESEARCH OBJECTIVES

Intensive apple cultivation systems and the use of dwarf rootstocks represent a modern solution for increasing production and profitability in fruit growing. The underlying hypothesis is based on the idea that the rootstock, through its influence on tree vigor, crown architecture, nutritional and water absorption efficiency, can lead to significant variations in:

1. Morpho-productive characteristics of the trees (vigor, precocity, yield per hectare, fruit-to-wood ratio);
2. Physical quality of apples (size, shape, volume, density, firmness);
3. Chemical composition of the fruits (sugar content, acids, vitamin C, phenolic compounds, antioxidant capacity);
4. Storage ability of apples under warehouse conditions (water loss, physiological degradation, preservation of firmness and chemical composition).

Therefore, in order to test this hypothesis, the following objectives were established and pursued:

1. Evaluation of tree growth – including the analysis of tree vigor depending on the cultivar–rootstock combination, covering tree height, trunk diameter, trunk cross-sectional area, annual shoot growth length, and crown volume.
2. Determination of yield per hectare – measurement of yield per hectare and per tree, followed by comparison of the production efficiency of different cultivar–rootstock combinations to identify the most economically efficient variants.
3. Determination of physical characteristics of the fruits (weight, dimensions, volume, density, flesh firmness) for six apple cultivars grafted on different rootstocks.
4. Analysis of the chemical composition of the fruits (soluble dry matter, total acidity, pH, water content, vitamin C, carotenoids, anthocyanins, polyphenols, and the antioxidant capacity of the fruits), depending on the cultivar and rootstock.

5. Determination of physico-chemical changes (water content, soluble dry matter, total acidity, pH, vitamin C) occurring during storage, based on cultivar and rootstock.

6. Analysis of the evolution of bioactive compounds (carotenoids, polyphenols, anthocyanins) during storage, in correlation with genetic factors (cultivar) and agrotechnical factors (rootstock used).

7. Evaluation of the antioxidant capacity of fruits at different stages of storage, depending on the cultivar–rootstock combination and the storage conditions applied.

SITE LOCATION AND ENVIRONMENTAL CONDITIONS

The experiment took place in the Republic of Moldova, Orhei District, Jora de Mijloc village (47°28′30″N; 29°05′43″E), at an altitude of 15 meters above sea level, during the 2018–2019 period. Jora de Mijloc is the administrative center of the commune with the same name, located in Orhei District, Republic of Moldova. The climate of Jora de Mijloc falls within the temperate continental zone, characterized by hot and dry summers and relatively mild winters, though occasionally affected by cold snaps. The location benefits from a climate favorable to agriculture, particularly for fruit growing and viticulture. The intensive orchard where the research was carried out was established in 2009, with a tree planting distance of 1–1.5 m x 4 m. The studied apple cultivars (Figure 1) were grafted onto M9 and MM106 rootstocks. The orchard covers an area of 216 hectares, and the planting material used to establish it was produced in the farm's own nursery. The trees were trained in a slender spindle form, typical of super-intensive plantations, and the annual maintenance pruning was performed according to "Zahn's rules." Six apple cultivars were included in this research, namely, 'Reinette Simirenko', 'Golden Delicious', 'Idared', 'Generos', 'Champion', 'Florina' (Figure 1). These cultivars, grafted on M9 and MM106 rootstocks, were all subjected to the same fertilization regime and phytosanitary treatments.

Fig.1. Apple cultivars under study
1-'Reinette Simirenko'; 2-'Golden Delicious'; 3-'Idared'; 4-'Generos'; 5-'Champion'; 6-'Florina'

1ST STUDY - THE INFLUENCE OF THE CULTIVAR AND ROOTSTOCK ON THE MORPHO-PRODUCTIVE CHARACTERS OF TREES

Aims

The main purpose of this study was to determine the influence of varieties and rootstocks on the development and production capacity of trees in a modern, intensive orchard.

Materials and methods

The intensive orchard where the research was carried out was established in 2009, with a tree planting distance of 1-1.5 m x 4 m. The varieties taken in the study were grafted onto rootstocks M9 and MM106.

The biological material used for this experiment consisted of six different apple cultivars: 'Reinette Simirenko', 'Golden Delicious', 'Idared', 'Generos', 'Champion', and 'Florina', grafted onto M9 and MM106 rootstocks. All were subjected to the same fertilization and phytosanitary treatments.

The orchard where the experiment was conducted covers an area of 216 hectares, and the planting material used for its establishment was produced in the family farm's own nursery.

The trees were trained in a slender spindle form, specific to super-intensive plantations, and the annual maintenance pruning was carried out following "Zahn's rules." Thus, scaffold branches formed on the central leader that were thicker than half the diameter of the leader, measured just below the branch, were removed. Branches in inappropriate positions were removed at the ring in the upper part of the tree, and with a short stub in the middle section, and a longer stub in the lower part to encourage the emergence of new, less vigorous lateral shoots, which in the future may develop into fruiting branches.

Results and conclusions

The results obtained showed significant differences between the analyzed apple cultivars across all evaluated parameters.

The trunk diameter values ranged from 98.05 to 107.8 mm among the six apple cultivars studied. The highest value was recorded for 'Generos' (107.80±0.03 mm), followed by 'Champion' (107.30±0.01 mm). The lowest value was measured in 'Reinette Simirenko' (98.05±0.07 mm), which was significantly different from the other experimental variants.

Measurements of tree height showed that the tallest and most vigorous trees belonged to the cultivar 'Golden Delicious' (3.36±0.06 m), followed by 'Florina' (3.21±0.02 m). The shortest trees were recorded for 'Champion' and 'Generos', with an average height of 2.80±0.08 m and 2.68±0.04 m, respectively.

The annual shoot length measurements indicated significant variation among the cultivars. The most vigorous cultivars in this regard were: 'Generos' with an average shoot growth of 30.87±0.28 cm; 'Champion' (28.37±0.26 cm); 'Florina' (26.87±0.09 cm) and 'Reinette Simirenko' (26.13±0.25 cm).

By monitoring and managing shoot growth, growers can improve orchard productivity, sustainability, and economic profitability.

Results from crown volume measurements indicated values typical of high-density orchards, ranging between 4.60 and 5.42 m^3. The smallest crown volume was recorded for 'Champion' (4.60±0.13 m^3), while the largest was in 'Golden Delicious' (5.42±0.18 m^3). Cultivars like 'Idared' (5.40±0.11 m^3), 'Reinette Simirenko' (5.36±0.13 m^3), and 'Florina' (5.37±0.16 m^3) had similar crown volumes.

Results regarding the trunk cross-sectional area (TCSA) highlighted 'Generos' with the highest value of 91.22±2.21 cm^2, while the lowest value was observed in 'Reinette Simirenko' (75.47±1.99 cm^2). Statistically significant differences were found among cultivars for TCSA,

although the differences between 'Champion' (90.38±2.13 cm^2) and 'Generos' (91.22±2.21 cm^2) were not statistically significant.

As for productivity, the most productive cultivars were 'Champion' with a yield of 54.46±1.57 t/ha; 'Idared' with 52.71±1.55 t/ha. Lower productivity was observed in 'Generos' (41.29±1.21 t/ha); 'Golden Delicious' (47.44 t/ha) and 'Florina' (47.44±1.36 t/ha). The lowest yield was recorded for 'Reinette Simirenko', with 43.04±1.25 t/ha.

The production efficiency of the studied apple cultivars ranged from 0.18 to 0.26 kg/cm^2, with the highest values in 'Idared' (0.26±0.03 kg/cm^2); 'Champion' (0.24±0.02 kg/cm^2) and 'Reinette Simirenko' (0.23±0.01 kg/cm^2).

2ND STUDY - INFLUENCE OF THE CULTIVAR AND ROOTSTOCK ON THE PHYSICAL CHARACTERISTICS OF FRUITS

Aims

The aim of this study was to evaluate and compare the influence exerted by variety-rootstock combinations on the physical characteristics of the fruits of six apple varieties.

Materials and methods

Apple cultivars grown in Jora de Mijloc, Orhei region, Moldova, were analyzed in this study. The studied varieties were grafted on rootstocks M 9 and MM 106. For each variety, 5 trees were selected, randomly chosen, in 3 repetitions (n = 15) and for fruit quality analysis, 100 apples/variety were harvested and analyzed. The measurements were carried out in the laboratory of the discipline of Fruit Growing and Biochemistry, within the Faculty of Horticulture and Business in Rural Development, Cluj-Napoca, with instruments and equipment appropriate to each character followed.

Results and conclusions

The results obtained in this study provide a detailed picture of how the variety and rootstock influence the physical characteristics of the fruits. Data analysis revealed significant variations between the tested combinations, demonstrating the importance of the correct choice of genetic material in optimizing fruit quality.

1. Significant Influence of Rootstock on the Physical Characteristics of the Fruits

Experimental data highlight the key role of the rootstock in shaping the physical parameters of the fruits (weight, size, volume, density, and firmness). Compared to MM106, the M9 rootstock resulted in smaller and lighter fruits, but with higher density and firmness—traits essential for post-harvest handling and storage.

2. Consistent Differences Between Cultivars on the Same Rootstock

The tested cultivars exhibited distinct physiological behaviors even when grafted on the same rootstock, confirming a cultivar–rootstock interaction with significant effects on fruit physical traits. For example, 'Champion' consistently produced larger and heavier fruits, regardless of the rootstock, while 'Florina' stood out with high firmness, suggesting good potential for long-term storage.

3. Firmness and Density: Parameters Correlated with Internal Quality and Post-Harvest Performance

Fruits from trees grafted on M9 had higher values for firmness and density, indicating a compact cellular structure with positive implications for handling and storability. In contrast, MM106 favored the development of larger fruits but with a softer internal texture.

4. Critical Role of Water Resources in Harnessing the Potential of MM106 Rootstock

While MM106 performs better in terms of water and mineral uptake and supporting vegetative growth, it requires careful water management to prevent declines in fruit physical quality under stress conditions. Without irrigation, the advantages provided by its moderate vigor may be compromised.

3RD STUDY - INFLUENCE OF THE CULTIVAR AND ROOTSTOCK ON THE CHEMICAL CHARACTERS OF FRUITS

Aims

The main purpose of this study is to evaluate the influence of the variety and the apple rootstock on the main chemical properties of the fruits, in order to identify the most advantageous variety-rootstock combinations from the perspective of the nutritional and functional quality of fruit production.

Materials and methods

The apple varieties analyzed in this study were grown in Jora de Mijloc, Orhei region, Republic of Moldova. The apples (100 fruits/variety) were harvested at commercial maturity, 150 days after flowering. The assessment of fruit maturity was based on the determination of epidermal colour, pulp firmness and soluble dry matter content, determined using a hand-held fruit penetrometer and a refractometer. The fruits were transported to the laboratory and stored at 4 °C, in relative humidity conditions of 80–90%.

The chemical analyzes were carried out in the laboratory of the discipline of Fruit Growing and Biochemistry within the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, according to the protocol specific to each parameter.

All chemicals and reagents used were of analytical quality, and the ultrapure water (18 MΩ cm) was treated in a Milli-Q water purification system. The carotenoid standards - carotene, lutein and zeaxanthin (98%, 95% and 98% purity, respectively) were purchased from Extrasynthese. All analyses were carried out after the apples were washed under tap water, and the skin was removed manually.

Results and conclusions

The study on the influence of M9 and MM106 rootstocks on the chemical composition of fruits in six apple varieties ('Golden Delicious', 'Reinette Simirenko', 'Florina', 'Idared', 'Champion' and 'Generos') highlighted significant intervarietal differences, as well as variety-rootstock specific interactions, reflected in the main biochemical and functional characteristics of the fruits, which led to the following conclusions:

The soluble dry matter (°Brix) was significantly higher in the varieties grafted on rootstock M9 ('Reinette Simirenko', 'Idared' and 'Florina') than those grafted on rootstock

MM106, which induced a slight dilution of sugars, especially in the less productive varieties.

Total acidity and pH showed clear variability between varieties. 'Reinette Simirenko' and 'Idared' stood out for their high acidity and low pH, associated with good post-harvest preservation, while 'Golden Delicious' and 'Generos' had a low acidity, favorable to the sweet and balanced taste. The MM106 rootstock favored a moderate balance between acidity and pH of the fruits, suitable for fresh consumption and processing.

The water content of the fruits was relatively homogeneous, but the varieties 'Reinette Simirenko' and 'Idared' stood out for higher values, correlated with juiciness and pleasant pulp texture. The M9 rootstock moderately influenced this characteristic, the trend being slightly downward in favor of the dry matter. The MM106 rootstock claimed a high water content in the fruits of certain varieties, such as 'Reinette Simirenko' (82.14%) and 'Golden Delicious' (80.93%), while 'Champion' and 'Florina' had lower values. This reflects the positive effect of the vigorous root system of MM106 on water absorption, but also the influence of pulp density, specific to each variety.

The vitamin C content varied significantly between varieties, with 'Reinette Simirenko' recording the highest content (11.60 mg/100 g), followed by 'Generos' and 'Champion' for both rootstocks.

The carotenoid profile of the analyzed varieties showed similar accumulation trends in both rootstocks, with maximum values recorded in the 'Idared' variety, with high levels of (all-E)-lutein, (all-E)-neoxanthin and β-carotene, indicating a higher antioxidant potential. 'Champion' presented a well-balanced profile, and 'Florina' was at the opposite pole, with the lowest carotenoid content.

The anthocyanin content was decisive for the colour of the fruit and was manifested only in the pigmented varieties in the case of amibolr rootstock used. 'Florina' had the highest cyanidin-3-O-galactoside content, followed by 'Idared' and 'Champion'. Both rootstocks supported the synthesis of bioactive compounds in combination with phenolic expressive genotypes.

The total phenol content was highest in 'Florin' (2723 mg GAE/kg), followed by 'Idared' and 'Champion'. This distribution was also reflected in the antioxidant capacity, where 'Florin' stood out clearly, followed by 'Idared' in the case of both rootstocks tested. The lower bioactive compound contents of 'Golden Delicious' and 'Reinette Simirenko' explain the low antioxidant activity values in these varieties.

The 'Florina' variety had the highest antioxidant capacity, correlated with the high levels of phenols and anthocyanins in both variants of rootstocks used. Rootstocks favored the accumulation of antioxidants in varieties with adequate genetic potential, but did not compensate for their lack in genotypes less productive from a phenolic point of view (e.g. 'Golden Delicious'). The results obtained in this research highlight the importance of carefully choosing the variety-rootstock combination in order to obtain apple fruits with high nutritional value and superior technological characteristics. In this context, the following recommendations can be made, relevant for manufacturers, researchers and industry alike:

Including phenolic compound content and antioxidant capacity in the labelling of processed apple products could help steer consumers towards healthier choices and boost the consumption of value-added fruits. Varieties with a complex biochemical profile ('Florina',

'Idared') can be promoted as natural sources of antioxidants in functional products, juices or healthy snacks. This integrated approach to the influence of rootstocks on the biochemical properties of apple fruits confirms the essential role of choosing the right graft-rootstock combination in obtaining high quality horticultural products, adapted to the requirements of the modern consumer and the requirements of the food industry.

4TH STUDY - INFLUENCE OF STORAGE CONDITIONS, CULTIVAR AND ROOTSTOCK ON FRUIT QUALITY

Aims

The main purpose of this study is to evaluate the combined influence of variety, rootstock and storage conditions on the physicochemical quality of apple fruits, in order to identify the most efficient technological combinations that allow maintaining the nutritional and commercial value of fruits in the post-harvest period.

Materials and methods

The chemical analyzes were performed at intervals of 30 days for 4 months in the laboratory of the discipline of Fruit Growing and Biochemistry within the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, according to the protocol specific to each parameter described above.

Results and conclusions

The results obtained in this study clearly highlight the decisive influence of the apple variety on the evolution of the physicochemical characteristics of the fruit during storage, followed, to a lesser extent, by the type of rootstock used and the shelf life.

1. The weight and volume of the fruits decreased significantly during the four months of storage, mainly due to water loss through sweating and breathing. The varieties 'Florina' and 'Idared' showed good morphological stability, especially on rootstock MM106, while 'Champion' was the most susceptible to dehydration.

2. The firmness has progressively decreased, most pronounced in the case of the 'Florina' variety, despite the high initial values. The decreases were smaller in 'Reinette Simirenko', 'Golden Delicious' and 'Generos', suggesting better textural strength.

3. The vitamin C content was sensitive to oxidation, with losses ranging from 10% to over 40%, depending on the variety. 'Golden Delicious' stood out for its superior stability, while 'Champion' and 'Reinette Simirenko' recorded rapid degradation.

4. The total acidity was dominantly influenced by the variety, with significant losses in the case of the varieties 'Florina' and 'Generos'. The varieties 'Golden Delicious' and 'Reinette Simirenko' preserved the acidic balance of the fruits better.

5. Soluble dry matter has decreased moderately in most cases, especially in varieties with a high initial content. 'Champion' and 'Generos' have shown good stability, which recommends them for storage or processing.

6. Phenolic compounds decreased by an average of 5–13%, depending on the variety. The varieties 'Florina', 'Champion' and 'Idared' showed good phenol retention during storage, which gives them a superior antioxidant potential.

7. Antioxidant activity (FRAP and ABTS) recorded reductions proportional to decreases in vitamin C and phenols. The most stable combinations were 'Champion'/M9 and 'Florina'/M9, with losses below 8%, which recommends them for fresh consumption with maintained functional benefits.

Therefore, it can be concluded that the variety plays a decisive role in the post-harvest period, and the rootstock indirectly influences biochemical parameters through changes in vigor, metabolism and tissue structure. The M9 rootstock generally conferred an antioxidant capacity and a more stable phenol content, while MM106 favored better weight and firmness preservation.

ORIGINALITY AND INNOVATIVE CONTRIBUTIONS OF THE THESIS

The originality of the research lies in its multidimensional character: on the one hand, the influences of the rootstock, the variety and the cultivation system on the growth and fruiting peculiarities of the trees (tree height, trunk diameter, trunk cross-sectional surface) are analyzed, and on the other hand, quality traits of the fruits are rigorously monitored – both fresh and during the storage period.

Through this holistic approach, the work makes a significant contribution to the understanding of how the choice of variety-rootstock combinations and the cropping system influences the fruiting performance of trees and the biochemical properties of fruits. One of the innovative contributions of the work is the direct correlation between the biochemical profile and the physical performance of fruits depending on the type of rootstock, providing a scientific foundation for the oriented selection of biological material according to the production destination (fresh consumption, storage, processing).

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