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EFFECT OF FERTILIZATION ON THE GROWTH, NUTRITIONAL AND PHYSIOLOGICAL STATUS OF APPLE PLANTING MATERIAL (*MALUS DOMESTICA* BORKH.) GROWN IN CONTAINERS

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Abstract

The study was conducted at the Fruit Growing Institute in Plovdiv to establish the influence of different fertilizer rates on the growth, nutritional and physiological status of apple plants of the Florina cultivar, grafted on M9 rootstock, grown in containers (10 l). Variants of the experiment are Variant I - Control (not fertilized), Variant II - $N_{1.3}P_{0.3}K_{0.7}Mg_{0.1}$, Variant III - $N_{2.7}P_{0.7}K_{1.3}Mg_{0.3}$ and Variant IV - $N_{4.3}P_{1.1}K_{2.1}Mg_{0.4}$. In fertilized variants, plants with a height of 144.11 to 152.14 cm and a stem diameter of 9.63 to 10.09 mm were obtained. The plants of the control variant reached average values for height of 92.38 cm and 6.95 mm for stem diameter. The plants of low fertilizer rate were distinguished by the largest volume of the root system (99.44 cm^3). There is a pronounced tendency for a direct increase in nitrogen with increasing fertilizer rate. The experimental data obtained showed that the low fertilizer rate ($N_{1.3}P_{0.3}K_{0.7}Mg_{0.1}$) was optimal and led to the production of high-quality apple planting material suitable for establishing fruit orchards.

***Key words**: apple, containers, fertilization, planting material.*

INTRODUCTION

The production of the necessary quantities of planting material is a basic prerequisite for the development of fruit growing. Quality planting material must be produced as it determines the future development of fruit trees.

One intriguing new approach in the production of fruit planting material is container production. This is a relatively new method of producing fruit plants and has many advantages (Haris & Gilman, 1993; Gilman & Beeson, 1996; Mathers et al., 2007). In this way, different types of fruit crops can be successfully grown.

This technology has the following two advantages: it allows almost 100% interception when planting in a permanent place, as planting does not cause stress to the newly planted plants and allows establishing orchards throughout the year (Akova, 2021).

Fertilization is a major agrotechnical practice, and is mandatory for the container growing (Akova et al., 2019; 2020a; 2020b).

Apple is a traditional fruit crop in Bulgaria. Florina is the most common apple cultivar in our country.

In Bulgarian fruit growing, conventional tree production in a nursery is mainly applied, which creates the need to explore the possibilities for producing apple planting material in containers.

The study aimed to determine the influence of fertilization on the growth, nutritional, and physiological status of apple planting material of the Florina cultivar produced in containers.

MATERIALS AND METHODS

In the period 2019-2021, a trial was held at the Fruit Growing Institute in Plovdiv to determine the influence of different fertilizer rates on the growth behavior, nutritional and physiological status of apple plants of the Florina cultivar grafted on M9 rootstock grown in containers.

The fertilizer experiment was set in four variants with ten replications, each plant being a separate replication.

The following variants were tested: Variant I - Control (not fertilized), Variant II - $N_{1.3}P_{0.3}K_{0.7}Mg_{0.1}$, Variant III - $N_{2.7}P_{0.7}K_{1.3}Mg_{0.3}$, and Variant IV - $N_{4.3}P_{1.1}K_{2.1}Mg_{0.4}$. The fertilization was applied several times with increasing rates of nutrients with the combined fertilizer Kristalon N (20%) - P_2O_5 (5%) - K_2O (10%) - MgO (2%). Optimal soil moisture was maintained in the containers, consistent with the requirements of the crop grown.

The following biometric indicators were reported: stem diameter (mm), plant height (cm), leaf area (mm^2), the volume of the root system (cm^3), and fresh mass of leaves, stems, and roots (g). Chemical analyses of leaf samples for the content of leaf pigments and nutrients were carried out, and the content of nitrogen, phosphorus, potassium, calcium, and magnesium was determined.

The leaf area was measured by scanning the leaves and analyzing the resulting images with specialized software (Gao et al., 2011). The volume of the root system was measured using Burdett's method (1979). The chlorophyll content (*a*, *b*, *a+b*) was determined spectrophotometrically in a 95% ethyl alcohol extract.

Table 1. Definitions of measured and calculated chlorophyll fluorescence parameters used in the experiment, based on Strasser and Strasser (1995) and Goltsev (2016)

Chlorophyll Fluorescence Parameters	Description
Measured parameters and basic JIP-test parameters derived from the OJIP transient	
$F_O \sim F20\mu s$	Minimum fluorescence, when all PSII reaction centres (RCs) are open; Fluorescence intensity at 20 µs
F_J	Fluorescence at the J-step (2 ms) of the O-J-I-P transient
F_I	Fluorescence at the I-step (30 ms) of the O-J-I-P transient
$F_M = F_P$	Maximum fluorescence at the P-step when all RCs are closed
$V_J = (F_J - F_O)/(F_M - F_O)$	Relative variable fluorescence at the J-step
$F_V = F_M - F_O$	Variable fluorescence
Quantum yields and probabilities	
F_V/F_M	Maximum quantum efficiency of PS II photochemistry
$\psi_{EO} = 1 - V_J$	Probability (at t=0) that a trapped exciton moves an electron into the electron transport chain beyond QA^-
$\varphi_{EO} = (1 - F_J/F_M)$	Quantum yield (at t=0) for electron transport from QA^- to plastoquinone
$\delta_{RO} = (1 - V_I)/(1 - V_J)$	Efficiency/probability (at t = 0) with which an electron from the intersystem carriers moves to reduce end electron acceptors at the PSI acceptor side
Performance indexes	
PI_{ABS}	Performance index of PSII based on absorption
$PI_{total} = PI_{ABS} \times \delta Ro/(1 - \delta Ro)$	Performance index of electron flux to the final PSI electron acceptors, i.e., of both PSII and PSI

Analysis of chlorophyll fluorescence was performed on the youngest fully developed leaves from the top of three representative plants of each variant. The main parameters of the rapid fluorescence of *chlorophyll a* were measured with a portable Handy PEA fluorimeter (Hansatech Instruments, UK). The analyzed sections of the leaves were darkened for 40 minutes with special clips. The induction curves of rapid chlorophyll fluorescence (OJIP test) were recorded for 1 s after illumination with a light pulse (3000 µmol m-2 s-1 PPFD).

Primary data processing was carried out using a PEA Plus program (V1.10, Hansatech Instruments Ltd., UK).

Measured and calculated parameters using this OJIP test (Table 1) were interpreted and normalized according to Strasser and Strasser (1995) and Goltsev (2016).

The results obtained were processed statistically using the method developed by David B. Duncan (Duncan, 1955; Harter, 1960). Software "R3.1.3", in combination with "RStudio-0.98" and installed package "agricolae 1.2-2" (Mendiburu, 2015).

RESULTS AND DISCUSSIONS

The mineral nutrition performed affects the growth manifestations of apple plants (Table 2). All fertilized variants (Var. II, Var. III, and Var. IV) had higher values at all measured characteristics compared to the control (Var. I). The differences were statistically proven.

Table 2. Effect of fertilization on the growth characteristics of apple planting material grown in containers

Variants	Stem diameter (mm)	Plant height (cm)	Root system volume (cm^3)	Leaf area (mm^2)
I	6.95 b	92.38 b	46.67 b	13,685.76 b
II	10.09 a	152.14 a	99.44 a	257,249.52 a
III	9.76 a	148.96 a	66.11 ab	190,959.12 a
IV	9.63 a	144.11 a	55.55 b	155,461.68 ab

The results obtained show that there were no significant differences in the main growth indicators between the fertilized variants (Var. II, Var. III, and Var. IV). The nourished plants (Var. II, Var. III, and Var. IV) had a height of 144.11 to 152.14 cm, and those of the control were characterized by lower average height values (92.38 cm) (Table 2, Figure 1).

The mean stem diameter in control plants was 6.95 mm. Fertilized plants (Var. II, Var. III, and Var. IV) had higher stem diameter values - from 9.63 to 10.09 mm. The differences to the non-fertilized control were statistically proven.

Figure 1. Growth habits of the experimental plants in the different variants at the end of the vegetation

Figure 2. Root system in the different variants

The tree root system volume was measured because, according to Jacobs and Seifert, 2004, this method does not lead to its violation and is relatively fast. Fertilization affects both the above-ground part of the plants and the root system (Akova et al., 2019, 2020a, 2020b, 2022). A significant increase was observed in the root system volume was observed (Table 2, Figure 2). The plants of the fertilizer variants (Var. II, Var. III, and Var. IV) had values from 55.55 to 99.44 cm^2, and those of the control variant were characterized by lower average values (46.67 cm^2). There were proven differences between the variants tested. The fertilizer rate is one of the factors that affects the quality of planting material. Excessively high fertilizer rates can cause growth depression. Variant II (99.44 cm^3) had the highest value for the volume of the root system. The medium and high rates (Var. III and Var. IV) inhibit root growth. The values for the volume of the root system in fertilized plants with the high rate were comparable with those in unnourished plants. A number of authors emphasize a positive correlation between root system volume and subsequent plant development under field conditions, with plants having larger root system volume showing higher survival (Rose et al., 1991a; 1991b; 1992; 1997; Jacobs et al., 2005).

A significant increase in leaf area has also been reported. The plants of the fertilized variants (Variant II, Variant III, and Variant IV) had leaf area ranging from 155461.68 to 257249.52 mm^2, and those of the control variant were characterized by lower mean values (13685.76 mm^2). The high fertilization dose (Variant IV) increased leaf area, but compared to the low and medium doses (Variant II and Variant III), the effect of fertilization was weaker.

The influence of different fertilizer rates on the content of the essential nutrients in the leaves is presented in Table 3.

Table 3. Content of essential nutrients in the leaves

Variants	N (%)	K (%)	P (%)	Ca (%)	Mg (%)
I	1.77	2.29	0.25	2.28	0.72
II	2.27	2.24	0.30	1.29	0.55
III	2.33	2.25	0.31	1.35	0.50
IV	2.35	2.35	0.33	1.23	0.50

During the 2019-2021 study, the applied fertilization had a beneficial effect on the macronutrient content in the leaves of the observed fruit species.

There was a pronounced tendency for a directly proportional increase in nitrogen with increasing fertilizer rate. In control plants, the nitrogen content was in the low supply range. The N content of fertilized plants was in the range of 2.27-2.35%, which corresponded to adequate supply, and was about 50% higher than that of the control plants (1.77%). The K content was in a range of high reserve, with the values of this nutrient being optimal in all variants.

The results regarding the P content showed a clear trend of increase with increasing fertilizer rate, and in all variants, the values for this nutrient were optimal.

The values for Mg content in leaves were higher in the control plants. The same trend was observed with regard to the concentration of *Ca*. This was probably due to the antagonism between the elements potassium and calcium. The application of increasing fertilizer rates of the element potassium leads to a lower absorbtion of *Ca* from plants. Despite this, the values for *Ca* in the leaves of fertilized plants were within optimal limits.

Table 4. Effect of fertilization on the chlorophyll content in the leaves

Variants	Chl. *a* mg/g (DW)	Chl. *b* mg/g (DW)	Chl. *a+b* mg/g (DW)	Carotenoides mg/g (DW)
I	1.50 b	0.54 b	2.05 b	0.54 b
II	3.54 a	1.10 a	4.64 a	1.18 a
III	4.17 a	1.40 a	5.57 a	1.49 a
IV	4.15 a	1.27 a	5.42 a	1.55 a

It has been found that the introduction of increasing concentrations of K and P leads to an increase in the rate of photosynthesis but does not significantly affect the chlorophyll content of the leaves (Wang et al., 2022). We observe such a trend in our study. From the experimental data obtained, it can be seen that statistically proven differences between the individual fertilization variants have not been found (Table 4). A higher content of *chlorophyll a* distinguishes plants of fertilizer variants. The *chlorophyll b* content was lower than that obtained for *chlorophyll a*, but a positive effect of feeding was observed in all fertilized variants. The results in the total *chlorophyll a+b* showed an increase in the values of the fertilization variants, suggesting a good adaptation of the plants to the growing conditions. The highest values for *chlorophyll a+b* were recorded when the medium fertilizer rate was applied (var. III) ($N_{2.7}P_{0.7}K_{1.3}Mg_{0.3}$). With an increase in the fertilizer norm, there was a gradual increase in the content of carotenoids. From the results obtained, we can conclude that the applied fertilization favors the accumulation and maintenance of chlorophyll in the leaves during the growing season, which leads to an improvement of the photosynthetic activity of the plants and their good adaptability to the growing conditions.
Although the content of photosynthetic pigments is not the only indicator of plant photosynthesis, their increase can be considered as an expression of a better structuring of the photosynthetic apparatus under conditions of improved nutrition.
Another indicator of the functional activity of plants' photosynthetic apparatus is chlorophyll fluorescence. The analysis of rapid fluorescence induction curves of *chlorophyll a* (OJIP test) links the structure and functionality of the photosynthetic apparatus. It allows a rapid assessment of plant viability, especially under stress (Strasser et al., 2000; 2004).

Table 5. Effect of fertilization on the biomass of leaves, stems, and roots of plants

Variants	FW		
	Leaves (g)	Stems (g)	Roots (g)
I	11.52 b	60.67 c	32.33 b
II	108.13 a	135.77 a	236.13 a
III	110.31 a	99.67 b	212.13 a
IV	98.76 a	89.52 b	206.58 a

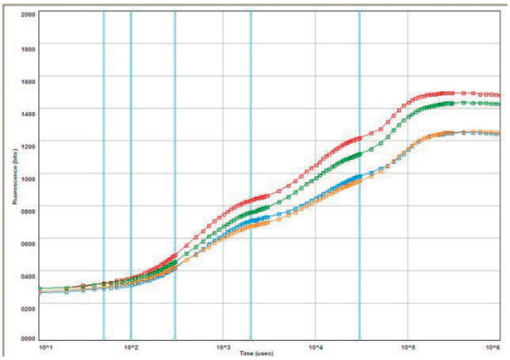

Figure 3. Induction curves of rapid chlorophyll fluorescence (OJIP test); (----) Control without fertilization; (----) variant II; (----) variant III; (----) variant IV

Table 6. Basic chlorophyll fluorescence parameters (JIP test) of apples grown in containers at different levels of fertilization

Variants/ Parameters	I (Control)	II	III	IV
F_0	294 a	305 a	328 a	319 a
Fm	1264 c	1273 c	1368 b	1447 a
Fv	970 c	969 c	1040 b	1128 a
Fv/Fm	0.763 b	0.790 a	0.750 b	0.775 a
ψE_0	0.546 a	0.599 a	0.492 a	0.585 a
φE_0	0.417 a	0.456 a	0.374 a	0.454 a
δR_0	0.484 a	0.519 a	0.493 a	0.457 a
PI abs	2.69 b	3.63 a	2.26 c	3.73 a
PI total	2.87 c	4.04 a	1.77 d	3.59 b

In the control and the three fertilizer variants, the rapid chlorophyll fluorescence curves had a typical F_0 to F_m level shape with pronounced J and I phases (Figure 3), indicating that the apple plants included in the experiment were photosynthetically active (Yusuf et al., 2010).

No significant differences were reported in the values of the minimum fluorescence (F_0) of control and treated plants (Table 6.). The highest value of the maximum fluorescence (F_m) was measured in variant IV, and the lowest was in the control. Despite fluctuations in initial (F_0), maximum (F_m), and variable (F_v) fluorescence, the quantum yield (F_v/F_m) reflecting the potential photochemical activity of photosystem II (PS II) ranges from 0.750-0.790 and corresponds to normal (0.750-0.830) in healthy, unstressed leaves (Bolhar-Nordenkampf & Oquist, 1993). This shows that a normally developed photosynthetic apparatus was functioning in all the studied variants. No statistically significant differences were reported for three important parameters of the JIP test – ψE_0, φE_0, and δR_0 in plants grown with and without fertilization. The Total Performance Index (PI total) reflects the functional activity of PS II and PS I and the electron transport chain between them. PI total is closely related to total plant growth and survival under stress and is considered a very sensitive indicator of the JIP test. The highest PI total was reported in variant II (4.04), which was significantly higher than in the control (2.87). The highest PItotal of the plants fertilized with the low dose (variant II) corresponded with their larger biomass, height, stem diameter, and leaf area and clearly showed the effectiveness of the applied treatment (Tables 2, 5, 6). Improved plant nutrition contributes to more efficient development and structuring of the photosynthetic apparatus, which in turn is a prerequisite for more intense photoassimilation and biomass accumulation. The lower values of some of the main chlorophyll fluorescence parameters (F_v/F_m, φE_0, ψE_0, PI abs, PI total) measured on the leaves of variant III are formally in contradiction with the more intensive plant growth of this variant compared to the control. This could be related to the growth rate of these plants - by the the time of measurement at the end of the vegetation, the shoots of the contro plants stopped their growth and formed an apical bud. The plants of variant III continued growing in height, and their top leaves (on which the measurement is made) were physiologically younger than those of the control ones.

CONCLUSIONS

All three fertilizer rates ($N_{1.3}P_{0.3}K_{0.7}Mg_{0.1}$, $N_{2.7}P_{0.7}K_{1.3}Mg_{0.3}$, and $N_{4.3}P_{1.1}K_{2.1}Mg_{0.4}$) significantly affect stem diameter and plant height growth resulting in apple planting material with larger sizes than the control (non-fertilized) trees.

The high fertilization rate ($N_{4.3}P_{1.1}K_{2.1}Mg_{0.4}$) inhibits root growth, and the values for the root system volume are commensurate with those of unnourished plants.

The fertilizer rate is one of the factors on which the quality of planting material depends. The low fertilization rate ($N_{1.3}P_{0.3}K_{0.7}Mg_{0.1}$) is optimal. It stimulates the growth and biomass accumulation, has a beneficial effect on the content of the essential nutrients and chlorophyll in the leaves, and leads to the production of high-quality apple planting material suitable for planting in orchards.

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