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IMPACT OF NITROGEN FERTILIZATION ON GROWTH AND PHOTOSYNTHETIC ACTIVITY OF WALNUT PLANTING MATERIAL (*JUGLANS REGIA* L.), CULTIVATED IN CONTAINERS

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Abstract

The object of the experiment was the walnut cultivar Izvor 10, grafted on a walnut rootstock (Juglans regia L.). The plants were propagated by the "Hot Callus" method and grown in containers (50 l) with peat-pearlite mixture (2:1). The impact of nitrogen fertilization on the growth and the physiological characteristics of young walnut plants was studied. Variants of the experiment were: Control (not-fertilized), Variant II - 2 g N / container and Variant III - 4 g N container. The height of the fertilized plants varied from 86 to 107 cm and the stem diameter - from 12.76 to 13.61 mm, while the control plants reached average values of 49.33 cm in height and 10.53 mm in stem diameter and the differences were statistically proven. It was found that fertilization with ammonium nitrate (NH_4NO_3), in the range of 2 - 4g N/container contributes to a more efficient development and structuring of the photosynthetic apparatus, which, on the other hand, is a prerequisite for more intensive photoassimilation and biomass accumulation. It was concluded that fertilization is mandatory for the production of walnut planting material in containers.

Key words*: walnut, pot cultivation, fertilization, vegetative characteristics, photosynthesis.*

INTRODUCTION

In the world of fruit growing the conventional production of planting material in the field is mainly applied which requires a lot of manual labour and is dependent on climatic and soil conditions. This is a prerequisite for the search of alternative approaches. One such approach is container cultivation, which has become increasingly popular in recent years. Its advantages are the easier to maintain the conditions of the cultivation, such as pH of the nutrient substrate, water and nutrient requirements, diseases and pests (Ruter, 1993). Plants grown in containers have a higher fine root mass than field-grown plants (Gilman & Beeson, 1996) and show much less stress when planted in the orchard (Harris & Gilman, 1993).

Fertilization is one of the most important practices for the quality of container grown plants, because they are grown in a limited nutritional volume which prevents their growth (Landis, 1989). According to Oliet et. al. (2004) fertilization can boost the growth of plants, improve their nutrient supply and increase the resistance to water stress, low temperatures and diseases.

Nitrogen is the most important nutrient in fertilizer programs because plants usually need more nitrogen during intense growth than other nutrients and is a key element in the applied fertilizers.

Often fertilizers used in plant nurseries with container cultivation exceed the required rates for optimal growth (Maust & Williamson, 1991).

Improving the efficiency of fertilizer application is one of the ways of reducing production costs and obtaining plants suitable for growing fruit orchards.

The aim of the present study was to investigate the impact of nitrogen fertilization on the growth and the physiological characteristics of the walnut plants grown in containers.

MATERIALS AND METHODS

The study was conducted in 2017 at the Fruit Growing Institute - Plovdiv, Bulgaria under the conditions of pot experiment. The object of the study was the walnut cultivar Izvor 10, grafted

on a walnut rootstock (*Juglans regia* L.). The plants were propagated by the "Hot Callus" method. The successfully grafted experimental plants in the winter months of January and February, 2017 were planted in March in plastic containers (3 l) with peat-perlite mixture (2:1; pH in the range of 5.5 to 6.5; N:P_2O_5:K_2O (14:16:18) and adapted to light in an experimental plot covered with an 80% shading net. Two weeks later, the already adapted plants were transferred to larger containers (50 l) and the 80% shading net was replaced with a 50% shading. The experiment was based on three variants in ten replications, each plant was considered a separate replicate.

Ammonium nitrate (NH_4NO_3) fertilizer was applied on the peat surface three times every twenty days, with the first application being made in mid-July. The following variants were thus formed:

I - Control (not fertilized);

II - 2 g N/container;

III - 4 g N/container.

The soil moisture in the containers was maintained to a field capacity, with the number of watering complied with the specific temperature conditions and the amount of precipitated rainfall.

Analysis of growth

At the end of the vegetation, the following parameters were taken into account: plant height (cm), stem diameter (mm), number of complex leaves. The leaves, stem and roots were separated and a specific fresh mass of the relevant botanical organs (g), leaf area (cm^2), root system volume (cm^3) were determined. The dry mass of the leaves, stems and roots was determined after drying at 80 °C to constant mass. The relative proportion of the individual botanical organs to the dry mass of the whole plant was determined as follows:

(Dry leaf mass/Dry mass of the whole plant) x 100 (%) (Leaf weight ratio, LWR)

The relative proportion of stems, roots and leaf stalks was calculated similarly.

The leaf area was measured by scanning the leaves and analysing the resulting images with specialized software (Gao et al., 2011).

The volume of the root system was measured by the Burdett method (1979).

Photosynthetic pigments

The content of chlorophyll (a, b, a+b) and carotenoids was determined spectrophotometrically in 95% ethyl alcohol extract (Skazkin et al., 1958).

Gas-exchange analyzes

Gas-exchange analysis was performed on the youngest fully developed leaves of 3 randomly selected plants of the respective variant. Measurements were taken with a LCpro + portable gasometer system (ADC, UK) on a sunny day at a light intensity of about 850 μmol m^{-2} s^{-1} PPFD and a temperature of 25 °C. Net photosynthesis rate (A, μmol CO_2 m^{-2} s^{-1}), transpiration intensity (E, mmol H_2O m^{-2} s^{-1}), stomatal conductivity (gs, mol $m^{-2}s^{-1}$) were determined.

Chlorophyll fluorescence

Chlorophyll fluorescence analysis was performed on the youngest fully developed leaves of 5 representative plants of the respective variant. The basic parameters of rapid chlorophyll fluorescence (JIP test) were taken with a HandyPEA portable system (Hansatech Instruments, UK). The leaves were dark adapted for 40 minutes with special clips. The main parameters of chlorophyll fluorescence were measured - minimal (F0), maximal (Fm), and variable (Fv) fluorescence, Fv/Fm, as well as HandyPEA-specific indicators - Performance index (PI_{ABS}) on an absorption basis and total PI (PItotal) measuring the performance up to the PSI end electron acceptors (Goltsev et al., 2010).

Statistical processing of data

The results obtained are subjected to mathematical analysis using the method developed by David B. Duncan (Duncan, 1955).

RESULTS AND DISCUSSIONS

The data presented in Table 1 shows the growth characteristics of walnut plants being influenced by the used nitrogen fertilizer rates. The fertilized variants (var. II and var. III) had higher values in all measured parameters compared to

the control (var. I), with the differences being statistically proven. The obtained results show that there are no significant differences between the fertilized variants (var. II and var. III) in the individual growth parameters.

Table 1. Influence of the nitrogen fertilization on the growth characteristics of walnut plants cultivar Izvor 10, cultivated in containers

Variants	Plant height (cm)	Stem diameter (mm)	Number of complex leaves	Leaf area (cm^2)	Volume of root system (cm^3)
I (Control)	49.33 b	10.53 b	17.00 b	4199.58 b	166.67 b
II	86.00 a	12.76 ab	24.33 a	11435.42 a	266.67 ab
III	107.00 a	13.61 a	26.00 a	13796.24 a	300.00 a

The nourished plants (var. II and var. III) had a height of 86 to 107 cm, and those of the control variant had lower average height values (49.33 cm) (Figure 1).

Figure 1. Appearance of the experimental plants at the end of the vegetation

The average stem diameter of the control plants was 10.53 mm. The fertilized plants (var. II and var. III) had higher average values for stem diameter - from 12.76 to 13.61 mm. The differences are statistically proven. As the fertilizer increased, the number of complex leaves, leaf area and volume of the root system increased significantly, with values at these growth parameters averaging 0.5 to more than 3 times higher than those of the control plants (var. I). Nourished plants (var. II and var. III) had higher average number of complex leaves, 24 to 26, and those of the control variant were characterized by lower average number (17).

The most significant increase was observed in the leaf area and the volume of the root system. The plants of fertilized variants (var. II and var. III) had leaf area from 11435.42 to 13796.24 cm^2, and those of the control variant were characterized by lower average values (4199.58 cm^2) (Figure 2).

Figure 2. Root system of the plants

The data shows that fertilization affects both the aboveground part of the plants and the root system. The average values of the volume of the root system of the nourished plants (var. II - 266.67 cm^3 and var. III - 300 cm^3) are higher than the non-nourished plants of var. I (166.67 cm^3). A number of authors point to a positive correlation between the volume of the root system and the subsequent crop development under field conditions, with higher root system planting material having higher survival rates (Rose et al. 1991a, 1991b, 1992, 1997; Jacobs et al., 2005).

Both fertilizer rates 2 g N/container and 4 g N/container are found to be effective and stimulate the growth of walnut plants.

Nourished plants (var. II and var. III) had higher average values of fresh and dry mass (leaves, stems, leaf stalks and roots), compared to control plants (var. I) (Table 2).

Table 2. Fertilization impact with ammonium nitrate (NH_4NO_3) on plants biomass

Variants	I (Control)	II	III
Fresh mass/leaves (g)	85.75 b	203.84 a	246.91 a
Fresh mass/stems (g)	55.10 b	110.41 ab	154.84 a
Fresh mass/roots (g)	243.82 a	309.52 a	314.60 a
Fresh mass/leaf stalks (g)	29.69 b	73.49 a	91.15 a
Dry mass/leaves (g)	28.76 b	73.81 a	87.20 a
Dry mass/stems (g)	26.73 b	44.20 ab	59.83 a
Dry mass/roots (g)	108.27 a	122.84 a	111.34 a
Dry mass/leaf stalks (g)	8.57 b	21.72 a	26.19 a

The fresh and dry mass of the leaves of the plants from the fertilized variants was twice as

high (203.84-246.91 g) as those of the control plants (85.75 g). The differences are statistically proven.

As the fertilizer rate increases, the average values of fresh and dry root mass also increases, but the differences being non-significant compared to the control.

The content of photosynthetic pigments in the leaves is an important indicator of the photosynthetic competence of the plants. The results obtained for the photosynthetic pigments in the leaves (mg/g fresh weight) are presented in Table 3.

Table 3. Content of photosynthetic pigments in leaves (mg/g fresh weight) of walnut plants with different doses of nitrogen

Variants	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Chlorophyll a + b (mg/g)	Carotenoids (mg/g)
I (Control)	1.22 c	0.53 a	1.75 b	1.43 ab
II	1.34 b	0.57 a	1.90 b	1.38 b
III	1.51 a	0.60 a	2.12 a	1.47 a

No symptoms of chlorosis were observed and the content of chlorophyll a was expected to be significantly higher than that of chlorophyll b for all variants tested. The concentration of chlorophyll a showed a considerable variation in the fertilization applied. As the fertilizer rate of nitrogen increased, the content of chlorophyll a also increased, and differences were statistically proven. There was a tendency towards an increase in chlorophyll b content with the increase of introduced nitrogen, but no statistically proven difference between the variants. The content of total chlorophyll (a + b) was the highest at the higher nitrogen fertilizer rate, and the difference with the control and the lower nitrogen dose is statistically proven. Nitrogen is a structural element of the chlorophyll and protein molecules and thus affects the formation of chloroplasts and the accumulation of chlorophyll in them (Tucker, 2004). Therefore, nitrogen fertilization has a direct effect on the chlorophyll content of the leaves of walnut plants. From the obtained results we can conclude that the high fertilizer rate has a favorable effect on the content of the plastid pigments in the leaves, which in turn is a prerequisite for their good photosynthetic competence. Although the content of photosynthetic pigments is not the only indicator for photosynthesis of plants, their increase can be considered as an expression of better structuring of the photosynthetic apparatus under conditions of improved nutrition.

The results of leaf gas exchange of walnut plants indicate that the increasing nitrogen dose had no significant effect on the rate of net photosynthesis (A) (Table 4). The significantly larger leaf area of the nourished plants results in an increased photoassimilation and significantly greater biomass accumulation.

Table 4. Effect of fertilization on transpiration intensity - /E/ (mmol H_2O $m^{-2}s^{-1}$), stomatal conductance - /gs/ (mol $m^{-2}s^{-1}$) and photosynthesis rate - /A/ (µmol CO_2 m^{-2} s^{-1}) at 850 µmol m^{-2} s^{-1} PPFD

Variants	Photosynthesis rate /A/ (µmol CO_2 $m^{-2}s^{-1}$)	Intensity of transpiration /E/ (mmol H_2O $m^{-2}s^{-1}$)	Stomatal conductance /gs/ (mol $m^{-2}s^{-1}$)
I (Control)	5.34 a	0.42 a	0.11 a
II	5.41 a	0.19 b	0.04 b
III	4.85 a	0.23 b	0.06 ab

Along with the intensity of photosynthesis, another indicator of the functional activity of the photosynthetic apparatus of plants is chlorophyll fluorescence. The analysis of the induction curves of rapid chlorophyll fluorescence (OJIP test) links the structure and functionality of the photosynthetic apparatus and allows rapid assessment of plant viability, especially in stress conditions (Strasser et al., 2000, 2004). In the three variants studied, the rapid chlorophyll fluorescence curves have a typical OJIP shape from F0 to Fm level with clearly separated J and I phases (Figure 3), indicating that the walnut plants included in the experiment are photosynthetically active (Yusuf et al., 2010).

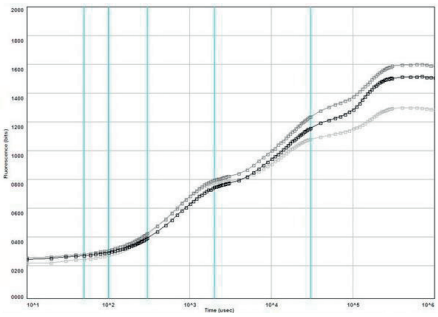

Figure 3. Induction curves of rapid chlorophyll fluorescence (OJIP test); (**XXX**) Control without fertilization; (**XXX**) variant II (2 g N / container); (**XXX**) variant III (4 g N / container)

The minimal (F_0) and maximal (F_m) fluorescence of the control plants was the lowest (Table 5), and the difference was statistically proven for F_0. At F_m, the values of the nourished plants are higher, but the difference is significant only in variant II. Lower F_m values may indicate that the photosynthetic object is in a state of stress and not all electron acceptors in PS II can be completely reduced. Maximal fluorescence is a complex parameter that is determined by a number of factors but also depends on the chlorophyll content of the tissues examined. Indeed, the lower Fm values in the control plants correspond to the measured lower content of total chlorophyll a and total chlorophyll in these plants (Tables 3 and 5).

Table 5. Basic parameters of chlorophyll fluorescence (JIP test)

Variants/Parameters	I (Control)	II	III
T for Fm	567 a	667 a	633 a
F0	242 b	283 a	276 a
Fm	1311 b	1610 a	1525 ab
Fv	1069 b	1326 a	1249 ab
F0/Fm	0.185 a	0.176 a	0.182 a
Fv/Fm	0.815 a	0.824 a	0.818 a
Fv/Fo	4.41 a	4.68 a	4.51 a
phi(E0)	0.39 b	0.49 a	0.50 a
psi(E0)	0.48 b	0.60 a	0.61 a
delta(R0)	0.42 a	0.45 a	0.46 a
PI abs	2.85 b	5.41 a	5.97 a
PI total	2.02 b	4.49 a	5.21 a

Despite fluctuations in the initial, maximum, and variable fluorescence, the quantum yield (Yield = F_v/F_m), reflecting the potential photochemical activity of PS II, ranges from 0.815-0.824 and corresponds to normal (0.750-0.830) in healthy, unstressed leaves (Bolhar-Nordenkampf and Oquist, 1993). This indicates that in all three variants studied, a normally developed photosynthetic apparatus was functioning. This is confirmed by the slight differences in the measured values of the rate of net photosynthesis. However, a more in-depth analysis of the parameters of the JIP test revealed some characteristic features of the potential of the photosynthetic apparatus in fertilized and control (unfertilized plants).

Characteristic differences between plants grown with and without N-fertilization were reported in the other three important parameters of the JIP test - psi (Eo) (ψEo), the performance index (PI abs) and the total performance index (PItotal). For plants cultivated on a nitrogen-enriched substrate, these parameters are higher and differences are statistically significant. ψEo reflects the probability of electron transport outside Q_A. The performance index (PI abs) shows the functional activity of the FS II relative to the energy absorbed, and the total performance index (PItotal) reflects the functional activity of the PS II, PS I and the electron transport chain between them. PI_{total} is closely related to overall plant growth and survival under stress and is considered to be a very sensitive indicator of the JIP test. The higher PItotal of the plants in the fertilized variants clearly shows the effectiveness of the applied treatment. Plant nutrition contributes to the more active development and structuring of the photosynthetic apparatus, which in turn is a prerequisite for more intensive photoassimilation and biomass accumulation (Table 2). No plants of the control variant reached the required size. The results obtained showed that both nitrogen norms (variant II and variant III) could well be used for production of planting material suitable for planting in orchards. Furthermore, in container grown walnut plants, fertilization should be a mandatory practice.

CONCLUSIONS

The fertilization with ammonium nitrate (NH_4NO_3) has a significant effect on the growth, the development of the photosynthetic apparatus and the construction of the biomass of the walnut plants grown in containers. Nutrition induces stronger growth compared to the control.

Both fertilizer rates 2 g N/container and 4 g N/container are effective, stimulate plant growth and are suitable for the production of walnut trees for planting in fruit orchards.

In container production of walnut plants, nutrition should be a mandatory practice.

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