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Growth and physiological performance of walnut plants grafted during winter dormancy and grown in containers

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Abstract. The study was carried out at the Fruit Growing Institute of Plovdiv (Bulgaria) during the period 2019-2022. The growth and physiological performance of walnut plants (*Juglans regia* L. 'Izvor 10') propagated during winter dormancy by the methods of cleft and whip/tongue grafting, grown in containers, were monitored, comparing them with those of walnut plants grafted following the standard technology of patch budding, grown in a nursery. The plants grown in a nursery were 120.4 to 122 cm in height and those grown in containers were characterized by lower average values – from 76.2 to 111 cm. The values for the stem cross-sectional area of the plants grown in the nursery varied from 349.9 to 379.7 mm^2 and they were higher than those grown in containers (from 88.2 to 221.8 mm^2). Higher values for the root system volume were reported for the plants grown in containers (283 – 294.4 cm^3), the differences being statistically significant. Plants grown in containers had a normally developed and functioning photosynthetic apparatus. No differences in chlorophyll fluorescence parameters were reported between patch-budded and container-grown plants.

Key words: walnut, grafted plants, vegetative behaviour, container growing, nursery, chlorophyll fluorescence.

Introduction

The ecological approach in the cultivation of agricultural crops is a global trend. It necessitates further profound studies and use of more plastic cultivars and new methods to follow the greening trends in modern agriculture. Such an approach is the container production of fruit planting material. It is a relatively new production method in Bulgaria and has many advantages – easier control of plant requirements for water and nutrients, protection from diseases and pests (Akova, 2021; Ruter, 1993). Container-grown plants had a higher fine root weight compared to field-grown plants (Gilman & Beeson, 1996). That approach saves the costs for agrotechnical measures (soil tillage and preparation) and the use of herbicides is limited.

Walnut is the major fruit species from the group of nut crops both worldwide and in our country, thanks to the rich nutritional and medicinal properties of walnut kernels (Nedev et al., 1983; Komanich, 1989; Germain et al., 1999).

It is known that walnut trees can be successfully used for prevention of landslides and in sloping terrains, for reducing wind velocity and wind erosion as a field protection belt, etc.

In 2022, the area occupied with walnut plantations in Bulgaria was 14 850 ha (Agrostatistics, 2022). Their maintenance, as well as the establishment of new orchards, makes it necessary to produce good quality walnut planting material.

The aim of the present research was to study the growth and physiological performance of walnut plants, propagated by cleft and whip/tongue grafting methods, grown in containers, comparing them with walnut plants, grafted following the standard technology of patch budding, grown outdoors in a nursery.

Materials and Methods

The experiment was carried out at the Fruit Growing Institute of Plovdiv (Bulgaria) during the period 2019 – 2022. The object of the study was the walnut cultivar Izvor 10, grafted on one-year-old common walnut (*Juglans regia* L.) seedling rootstock. The experiment was carried out in three variants with twenty-five replications, each plant being a separate replication (Fig. 1).

The experimental variants were as follows:

Var. I. Plants grafted by patch budding during the growing season and grown outdoors in a nursery (Control);

Var. II. Plants grafted during the winter dormancy by the method of cleft grafting and grown in con-tainers under 50% sun protection;

Var. III. Plants grafted during the winter dormancy by the method of whip/tongue grafting and grown in containers under 50% sun protection.

The plants in Var. I were grown in the open, grafted by the adopted patch budding method (Nedev et al., 1976) and those in Var. II and Var. III were propagated in a stratification room and planted in 10 L containers in a mixture of peat and perlite (2:1), (Fig. 2). All the grafted experimental plants were grown for two vegetation seasons in a shaded field and fed with a combined fertilizer N:P:K – 20:5:10, MgO – 2%, SO_3 – 25% + micro elements (Fe – 0.07%, Mn – 0.04%, Mo – 0.004%, Zn – 0.025%, B – 0.025%, Cu – 0.01%) at a rate of 1.3 g/L substrate.

Patch budding in a nursery
Var. I

Cleft grafting
Var. II

Whip/tongue grafting
Var. III

Fig.1. Walnut propagation using different grafting methods.

Growing grafted plants in a nursery
(Var I., Control)

Growing grafted plants in containers
(Var. II and Var. III)

Fig. 2. Photos of growing grafted walnut plants.

The soil moisture in the containers was maintained up to the maximum field moisture content, the irrigation rate being determined according to the specific temperature conditions and the amount of precipitation.

The following biometric parameters were recorded: tree height (cm), stem cross-sectional area (mm^2), root system volume (cm^3) and the main parameters of chlorophyll fluorescence.

The chlorophyll content in the leaves was measured with a chlorophyll content meter CL-01 (Hansatech Instruments Ltd., UK). The field-portable hand-held device determines the relative chlorophyll content (in relative units) using dual-wavelength optical absorbance measurements (660 nm and 940 nm wavelength) from leaf samples.

Chlorophyll fluorescence (OJIP test)

For a more detailed assessment of the physiological state of the plants, an analysis of chlorophyll fluorescence was performed. Plant Efficiency Analyser (Handy-PEA, Hansatech Instruments Ltd., UK) was used to analyse the structure and functional state of the photosynthetic apparatus, in order to detect early symptoms of stress and various disorders – OJIP test (Strasser et al., 2000, 2004). Measurements were taken on the first fully developed leaf from the tip (3rd leaf). Measuring the leaf chlorophyll content and the OJIP test were performed on the same leaf petioles in the middle part of the compound leaf. Five plants from each variant were measured. The analysed leaves` spot areas were adapted to dark with special clips for 40 minutes. The induction curves of the rapid chlorophyll a fluorescence (OJIP test) were recorded after illumination with 3000 µmol $m^{-2} s^{-1}$ PPFD for 1 s. The primary data were processed with the PEA Plus Software (V1.10, Hansatech Instruments Ltd., UK). The parameters of the OJIP test (Table 1) were interpreted and normalized according to Strasser & Strasser (1995) and Goltsev et al. (2016). The method is non-destructive and is applied without damaging or destroying the analysed plants.

The obtained results were processed statistically by Duncan's test (Steele & Torie, 1980), through the program "R studio" (R Core Team, 2020), using the packages 'agricolae' (Mendiburu, 2021), "readxl" (Wickham, 2019), "Rcpp" (Eddelbuettel & Balamuta, 2018) and "rstatix" (Kassambara, 2021).

Fig. 3. Chlorophyll fluorescence reading.

Table 1. Measured and calculated chlorophyll fluorescence parameters used in the experiments (Strasser & Strasser, 1995; Goltsev et al., 2016)

Chlorophyll fluorescence parameters	Description
$F_O \sim F20\mu s$	Minimum fluorescence when all reaction centres (RCs) of photosystem II (PSII) are open – at 20 μs
F_J	J-step fluorescence (2 ms)
F_I	I-step fluorescence (30 ms)
$F_M = F_P$	Maximum fluorescence (P-step) when all RCs are closed.
$V_J = (F_J - F_O)/(F_M - F_O)$	Relative variable fluorescence of J-step
$F_V = F_M - F_O$	Variable fluorescence
Quantum yields and probabilities	
$\psi_{EO} = 1 - V_J$	Reoxidation efficiency of Q_A- (electron transfer to $Q_B)^-$
$\varphi_{EO} = (1 - F_J/F_M)$	Quantum yield (at t = 0) for electron transport from Q_A^- to plastoquinone
$\delta R_O = (1 - V_I)/(1 - V_J)$	Efficiency/probability of electron transport from PSII to PSI
Performance indices	
PI_{ABS}	PSII performance index on absorption basis
$PI_{total} = PI_{ABS} \times \delta Ro/(1 - \delta Ro)$	Total performance index reflecting electron transport from PSII to PSI and ETV, i.e. measure of FSA performance all the way to final FS I acceptors

Results

The results of the growth performance of the experimental plants, grafted by the different methods, are presented in Table 2. The obtained results show that there were statistically significant differences in the major growth characteristics between the plants grown according to the standard technology in a nursery (Var. I) and those grown in containers (Var. II and Var. III).

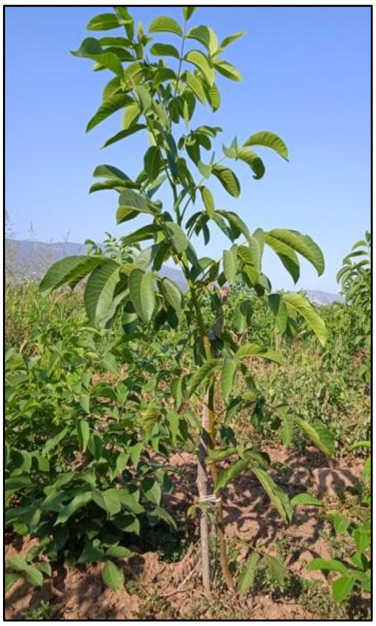
The plants grown in a nursery (Var. I) had higher values for height and cross-sectional area of the stem compared to those grown in containers (Var. II and Var. III), (Fig. 4).

The cleft-grafted plants (Var. II) had higher values of the cross-sectional area compared to those grafted by the method of whip/tongue grafting (Var. III), a statistically significant difference being reported only in 2021.

A significant increase in the root system volume was found during the period 2019-2020 in the plants grown in containers compared to the plants grown in a nursery and the differences being statistically significant (Fig. 5). In 2021, the results obtained for the root system volume varied within a narrower range. A lower average value was reported for the plants grown in a nursery com-pared to the plants grown in containers but there was no statistically significant difference between the variants.

Table 2. Vegetative performance of grafted walnut plants during the period 2019-2021.

VARIANT	2019	2020	2021	Average for the period
Plant height, cm				
I. Patch-budded plants in a nursery (Control)	122.0 a	121.2 a	120.4 a	121.2 b
II. Cleft-grafted plants	80.1 b	95.5 b	111.0 b	95.6 b
III. Whip/tongue grafted plants	76.2 b	89.8 b	103.4 b	89.8b
Stem cross-sectional area, mm^2				
I. Patch-budded plants in a nursery (Control)	349.9 a	355.4 a	379.7 a	361.6 a
II. Cleft-grafted plants	103.8 b	163.3 b	221.8 b	163.0 b
III. Whip/tongue grafted plants	88.2 b	137.3 b	183.5 c	136.3 b
Root system volume, cm^3				
I. Patch-budded plants in a nursery (Control)	166.0 b	219.6 b	273.0 a	219.5b
II. Cleft-grafted plants	286.0 a	294.4 a	302.8 a	294.4 a
III. Whip/tongue grafted plants	283.0 a	292.4 a	302.0 a	292.4a



A plant grafted by patch budding in a nursery
Var. I. (Control)

A plant grafted by the method of cleft grafting
Var. II

A plant grafted by the method of whip/tongue grafting
Var. III

Fig. 4. Walnut plants grafted by different methods.

The root system of a grafted plant grown in a nursery

The root system of a grafted plant grown in a container

Fig. 5. Root systems of grafted walnut plants grown under different conditions.

Indeed, the most vigorous growth in height, the largest number of leaves and the highest fresh and dry weight of the leaves were reported in the Control (Table 3). Concerning the other biometric characteristics, statistically significant differences were found again between the plants grown in containers and outdoors in a nursery. In the Control variant, higher values were reported for thickness, number, fresh and dry weight of the root system and the stem. Higher values were found for the chlorophyll content in the walnut planting material grown in containers compared to the Control variant.

Table 3. Biometric characteristics of grafted walnut plants.

Characteristics	Patch-budded plants in a nursery Var. I (Control)	Cleft-grafted plants Var. II	Whip/tongue grafted plants Var. III
Height, cm	121.22 a	95.54 b	89.82 b
Width, mm	21.26 a	14.40 b	13.2 b
Root system volume, cm^3	219.6 b	294.4 a	292.4 a
Number of compound leaves	23.8 a	21 ab	20.4 b
Fresh weight of leaves, g	224.82 a	126.94 b	115.56 b
Dry weight of leaves, g	75.96 a	43.62 b	39.68 b
Leaf area, mm^2	655510.9 b	789903.6 a	603991.1 b
Fresh weight of stem, g	222.14 a	112.24 b	112.26 b
Dry weight of stem, g	118.5 a	64.52 b	64.42 b
Fresh weight of root, g	388.24 a	368.28 a	278.44 b
Dry weight of root, g	167.76 a	156.6 a	113.68 b
Chlorophyll, relative units	9.98b	15.92 a	14.9 a

The parameters calculated by the OJIP test allow the quantification of the efficiency of absorption and utilization of light energy by the electron transport chain of photosynthesis. In addition to assessing the functionality of photosystem II (PS II), it reflects the rate of electron transport in the thylakoid membrane and the subsequent processes of functioning of ferredoxin-NADP oxidoreductase and the Calvin Cycle (Schansker et al., 2003).

The induction curves of rapid chlorophyll fluorescence in all the three studied variants had a typical OJIP shape from F0 to FM level, with clearly separated J and I phases (Fig. 6), indicating that the analysed walnut plants were photosynthetically active (Yusuf et al., 2010). The minimum fluorescence (F0) of the control plants (patch-budded) and those grafted by whip/tongue and cleft grafting methods did not differ significantly (Table 4). The maximum (FM) and variable fluorescence of plants from the control variant were lower compared to the two studied variants.

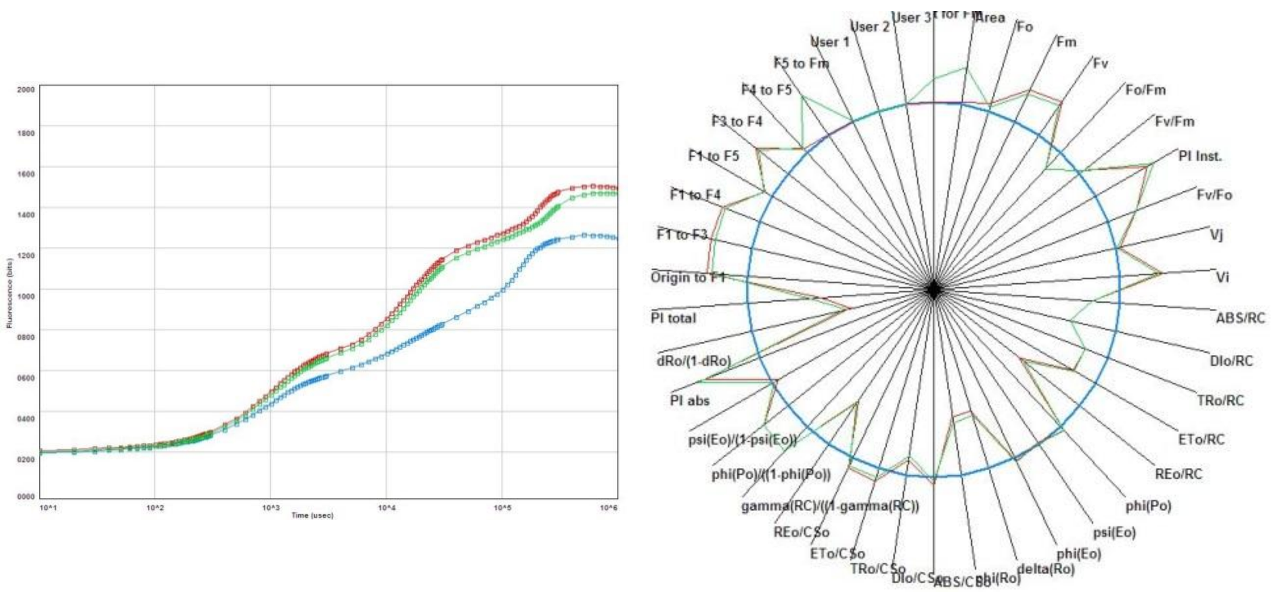


Fig. 6. Induction curves of rapid chlorophyll fluorescence (OJIP test) – (left) and radar diagram of the parameters (right) of walnut plants grafted in 2020 by different methods: **patch budding**; **whip/tongue grafting**; **cleft grafting.**

Table 4. Main parameters of chlorophyll fluorescence (OJIP test) in walnut plants grafted in 2020.

Parameter	Patch-budded plants in a nursery Var. I (Control)	Cleft-grafted plants Var. II	Whip/tongue grafted plants Var. III
F_0	230 a	234 a	240 a
F_M	1277 b	1481 a	1513 a
Fv	1047 b	1247 a	1273 a
Fv/F_M	0.818 b	0.842 a	0.842 a
ψE_0	0.679 a	0.677 a	0.671 a
φE_0	0.556 a	0.567 a	0.565 a
δR_0	0.604 a	0.422 b	0.406 b
PI_{abs}	9.75 a	13.69 a	13.25 a
PI_{total}	14.60 a	10.31ab	9.47 b

Discussion

A number of authors pointed out a positive correlation between the root system volume and the subsequent development of the plants under field conditions, the planting material with a larger root system volume having a higher survival rate (Rose et al. 1991a,b, 1992, 1997; Harris & Gilman, 1993; Vizzoto et al., 1993; Gilman, 2001; Jacobs et al., 2005; Mathers et al., 2007; Hoskins et al., 2014;). Ruter (1993) noted that the root system was not damaged when the plants were taken out of the containers and they had a higher fine root weight compared to those grown in the field (Fig. 5).

The parameters calculated by the OJIP test allow the quantification of the efficiency of absorption and utilization of light energy by the electron transport chain of photosynthesis. In addition to assessing the functionality of photosystem II (PS II), it reflects the rate of electron transport in the thylakoid membrane and the subsequent processes of functioning of ferredoxin-NADP oxidoreductase and the Calvin Cycle (Schansker et al., 2003).

Regardless of the fluctuations of F0, FM and FV, the quantum yield (FV/FM) reflecting the potential photochemical activity of photosystem II (PS II), ranged from 0.818 to 0.842 and corresponded to the normal limits (0.750-0.830) for healthy, unstressed leaves (Bolhar-Nordenkampf & Oquist, 1993). It showed that a normally developed photosynthetic apparatus was functioning in the plants produced by all the three grafting methods. No differences were reported between the control and the other two grafted variants in two important parameters of the OJIP test – φE0, ψE0 (Table 4). ψE0 parameter reflects the probability of electron transport outside the primary electron acceptor QA and φE0 – the quantum yield of electron transport from QA- to plastoquinone. δR0 values reflecting the efficiency of non-electron transport from PSII to PSI, were higher in the Control variant. The highest value of PI total (14.6) was also reported in the Control. That characteristic reflects the functional activity of PS II, PS I and the electron transport chain between them. The higher values of PI_{total} in the control variant reflect the more intensive growth and accumulation of biomass in the plants grown in the nursery in the open field. P_{Itotal} is closely related to general plant growth and survival under stress and is considered a very sensitive parameter of JIP test (Goltsev et al., 2016).

Conclusion

It is possible to produce walnut planting material in containers, propagated during winter dormancy by cleft and whip/tongue grafting methods. Patch-budded plants, grown in a nursery, were higher and had a larger cross-sectional area of the stem compared to the plants cleft-grafted during winter dormancy and grown in containers. The root system volume of the plants grown in containers was larger compared to those grown in a nursery. Cleft and whip/tongue grafting methods did not lead to differences in the vegetative performance of plants grown in containers. A normally developed photosynthetic apparatus was functioning in the plants propagated during winter dormancy by cleft and whip/tongue grafting methods and grown in containers.

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