

RESEARCH ON THE GROWTH AND DEVELOPMENT OF PLANTING MATERIAL FOR THE WALNUT GRAFTED USING THE CHIP BUDDING METHOD

CERCETĂRI PRIVIND CREȘTEREA ȘI DEZVOLTAREA MATERIALULUI SĂDITOR LA NUCUL ALTOIT PRIN METODA CHIP BUDDING

FLOREA M.¹, ISTRATE M.^{1}, APOSTOL Maria¹*

*Corresponding author e-mail: mistrate2000@yahoo.com

Abstract. *The determinations aimed to study the growth and development of four walnut varieties (Anica, Grădinari, Miroslava and Velnița) grafted using the Chip budding method. Biometric observations focused on the percentage of grafting success, and the height and diameter of the trees. In addition to biometric observations, biochemical determinations were performed on the content of photosynthetic pigments and peroxidase activity for the grafted combinations in the first year of vegetation. The research results highlighted differences between the four walnut varieties in terms of growth vigor, photosynthetic pigment content and peroxidase activity.*

Key words: grafting, peroxidases, walnut, photosynthetic pigments

Rezumat. *Determinările au avut ca scop studierea creșterii și dezvoltării a patru soiuri de nuc altoit (Anica, Grădinari, Miroslava și Velnița) prin metoda Chip budding. Observațiile biometrice au vizat procentul de prindere la altoire, înălțimea și diametrul pomilor. Pe lângă observațiile biometrice s-au efectuat și determinări biochimice privind conținutul în pigmenți fotosintetici și a activității peroxidaze la combinațiile altoite aflate în primul an de vegetație. Rezultatele cercetărilor au scos în evidență diferențe între cele patru soiuri de nuc privind vigoarea de creștere, conținutul în pigmenți fotosintetici și activitatea peroxidazică.*

Cuvinte cheie: altoire, peroxidaze, nuc, pigmenți fotosintetici

INTRODUCTION

The production of grafted walnut involves a multitude of organizational and cultural operations that include the production and preparation of the graft and rootstock for grafting, planting and handling the plants in the nursery (Tsurkan, 1990). The success of obtaining grafted walnuts under controlled environmental conditions depends on the choice of grafts and rootstocks (Mitrović *et al.*, 2008), and the time when the graft

¹University of Agricultural Sciences and Veterinary Medicine Iasi, Romania

branches are harvested (Paunović *et al.*, 2012). Research in the field shows that the success of walnut grafting is influenced both by the chosen grafting method (Solar *et al.*, 2001; Tshering *et al.*, 2006) and by the relative climatic factors inside the callus chamber (air temperature and humidity) (Avanzato, 2009; Barut, 2001). All these factors cumulated with the environmental conditions and the application of technological works specific to growing trees in the nursery lead to a success rate of up to 50-60% of the total number of grafted rootstocks (Paunović, 2010). The results from the literature suggest for obtaining a higher percentage of grafting, the need to produce the grafted walnut under controlled conditions and covering the grafting point with paraffin and polyethylene foil.

Paunović MS *et al.*, 2012 demonstrate the need to use foliar treatment as an essential practice of plant care, especially during bad weather, to obtain both a high percentage of grafting success and to improve the vegetative growth of the plants in the nursery.

In Romania, Achim and Botu (2001) demonstrated that the open field propagation of grafted walnuts is possible in the climatic conditions present in the Carpathians area. The results obtained from their studies showed that the best time for budding was from May 15 to June 15, the grafting being done using buds collected during the dormancy period of trees, that were kept in the refrigerator at a temperature of 1-4 °C. The success rate was influenced both by the type of rootstock and the time of cutting after budding. The planting of the rootstocks (*J. regia* L.) was done at the beginning of spring, their forcing and grafting was done in the same year in the optimal period mentioned above, with a percentage of grafting success of 78.0%. In the case of planting rootstocks during the previous year and grafting in the same period of time, the percentage of grafting success decreased to 40.0%.

In both cases, cutting the rootstock immediately after budding reduced the percentage of grafting success. Therefore, it is recommended that rootstocks be cut fifteen days after budding.

According to the authors, the climatic conditions in Romania present the risk of frost when propagating walnuts in the open air and that is why it is important that the production of grafted walnuts be carried out under controlled environmental conditions.

MATERIAL AND METHOD

In order to achieve the proposed objectives, in 2017 and 2018, on the experimental field from Bălțați commune, Iași county, the experiment was organized in protected areas. The experiment was organized in four experimental plots that were

structured according to the method of randomized blocks, the tree growing being done in solariums. Four walnut varieties (Anica, Grădinari, Miroslava and Velnița) were studied and grafted on a rootstock represented by a local biotype using the Chip budding method.

During the studies, biometric measurements were performed to assess the percentage of grafting success; also, measurements were done to determine the height of the trees, the diameter in the grafting area and at 1m above the grafting area. The laboratory analyses aimed to quantify the content of photosynthetic pigments in the leaves of the four walnut varieties studied and to determine the peroxidase activity in the leaves during the vegetation period.

The extraction and determination of the assimilatory pigments were carried out in accordance with the Current Protocols in Food Analytical Chemistry (Lichtenthaler and Buchmann, 2001). The tissues of the fresh leaves (0.1 g) were ground in the mortar in acetone (pure solvent) and then centrifuged at 10000 x g for 5 minutes. After centrifugation, the reading of the supernatant was done at the absorbance of 661.6 nm for chlorophyll a (Chl. a), at 644.8 nm for chlorophyll b (Chl. b) and at 470 nm for carotenoids (car.), using the T70 UV/VIS Spectrophotometer PG. The peroxidase (POD) activity was estimated according to Artenie et al. (2008).

RESULTS AND DISCUSSIONS

The grafting method and the time in which it is performed influence the percentage of grafting success (Achim and Botu, 2001; Razee *et al.*, 2008; Soleimani *et al.*, 2010). In addition, the following factors have a major importance in obtaining a high percentage of grafting success in walnuts: the amount of hormones, phenolic acids, juglone, starch, nitrogen, soluble sugars and other factors in different cultivars (Rongting and Pinghai, 1990).

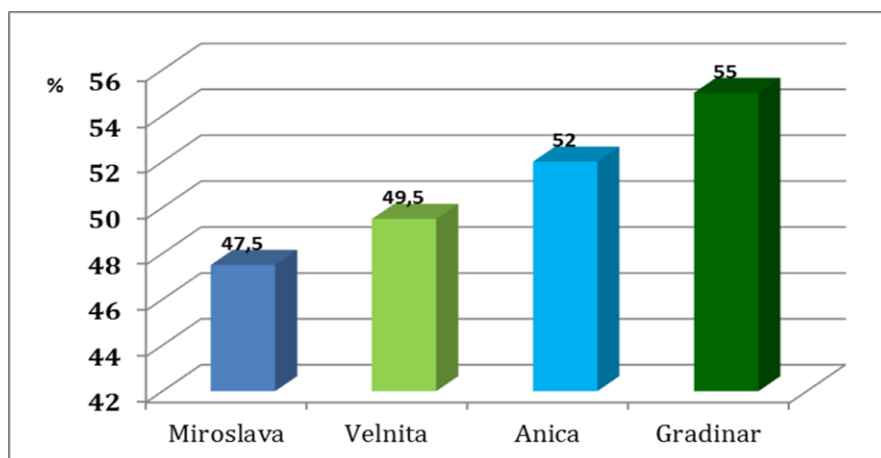


Fig. 1 Percentage of grafting success using the chip budding method %

The results regarding the average percentage of grafting success show higher values in the case of the Grădinari (55%) and Anica (52%) varieties and lower values in the case of the Miroslava (47.5%) and Velnița (49.5%) varieties.

Morphometric determinations revealed a significant difference in tree vigor for the four walnut varieties grafted using the chip budding method. Regarding the growth vigor of the trees in the second field of the nursery, this is given by the increase in height of the trees and their diameter at the level of the grafted area.

The comparison of the results obtained regarding the height of the grafted trees for the four grafted walnut varieties (fig. 2) shows the highest increase in height for the Anica variety, 209 cm, and the lowest height for the Velnița variety, 177 cm.

The other two varieties, Miroslava and Grădinari, had an average tree height of 192 cm.

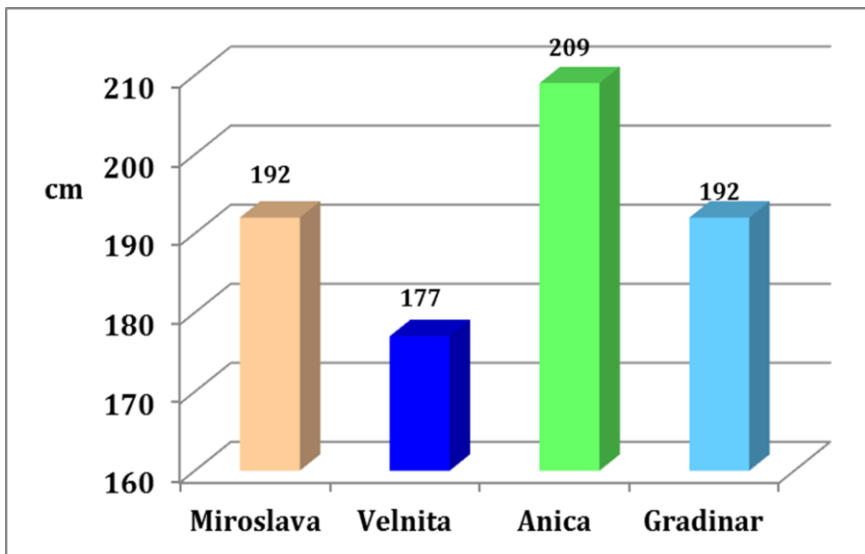


Fig. 2 Height of the trees grafted using the chip budding method (cm)

An important indicator in assessing the quality of trees obtained by grafting is the diameter of the trees. The growth vigor of the trees was highlighted by determining the diameter of the trees at the grafting point and at 1 m above the grafting point. After comparing the results, it is found that the Anica variety has the largest diameter, as in the case of plant height. In the case of the Velnița variety, the growth vigor was lower, presenting the smallest height as well as diameter among the four grafted varieties. The other two varieties (Miroslava and Grădinari) recorded similar values in terms of the diameter of the trees at the grafting point and 1 m above the grafting point.

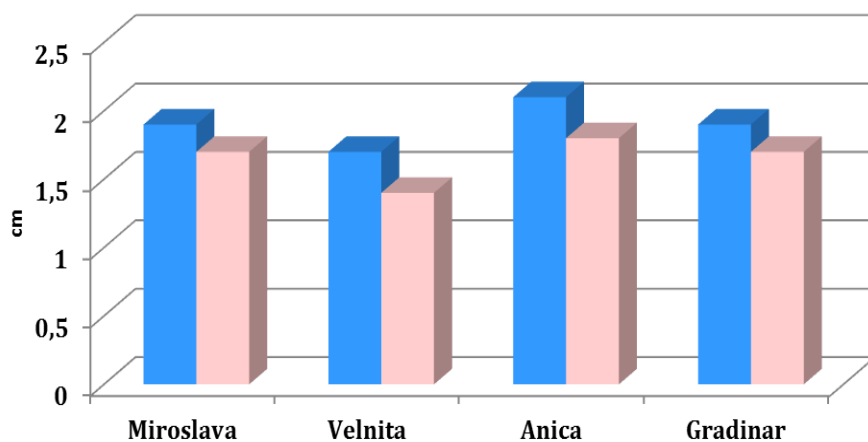


Fig. 3 Diameter of the grafted trees (cm)

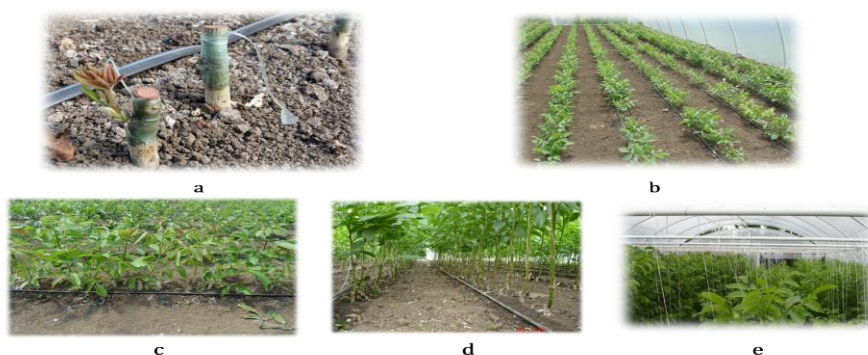


Fig. 4 Phenological aspects of the experiment

Table 1

Average content of photosynthetic pigments (mg / g D.W)

walnut	Cl. a mg/g D.W	Cl. b mg/g D.W	x+c mg/g D.W	Σ	Chl./Car. ratio	Chl.a/ Chl.b
Miroslava	2,01±0.03	0,65±0.03	0,67±0.02	3,33	3,97	3,09
Velniț a	2,1±0.035	0,70±0.04	0,75±0.05	3,55	3,73	3,00
Anica	2,25±0.03	0,72±0.03	0,74±0.04	3,71	4,01	3,13
Grădinari	2,21±0.06	0,67±0.06	0,69±0.03	3,57	4,17	3,30

± d = standard deviation

The main pigments of the leaves, the content of chlorophylls and carotenoids provide valuable information about the physiological status of the plants. These pigments are closely related to the manifestation of stress in plants, changes in their content highlighting the physiological state during their development and the adaptation of plants to different stress conditions (Demmig-Adams *et al.*, 1996).

For the four grafted walnut varieties, the chlorophyll pigment content varied between 3.71 mg/g s.u. and 3.33 mg/g s.u. in the case of the Miroslava variety (tab. 1). By comparing the results, it is noted that the highest values of chlorophyll pigments were obtained for the varieties with the best percentage of grafting success (Anica and Grădinari).

According to Fang *et al.*, 1998, stress conditions and changes in the abiotic factors cause a decrease in chlorophyll content and the chlorophyll a / chlorophyll b ratio. For the walnut varieties studied, the chlorophyll a / chlorophyll b ratio was within the theoretical limits, varying between 3.00 mg/g s.u. for the Miroslava variety and 3.30 mg/g s.u. for the Grădinari variety.

Another important indicator that reflects the physiological stress caused by the anatomical, physiological and genetic differences manifested between grafts and rootstocks is the decrease of the chlorophyll / carotenoid pigments ratio. The literature theoretically indicates a value of 4.8: 1 of the chlorophyll / carotenoid pigments ratio, the values being between 4.5-5 for plants grown on sunny terrains and 5.5-7 for plants grown on shaded areas. The results of the studies show that this ratio was between 3.73 mg/g s.u. for the Velniț a variety and 4.17 mg/g s.u. for the Grădinari variety. The obtained results highlight a normal development of the four walnut varieties grafted using the chip budding method.

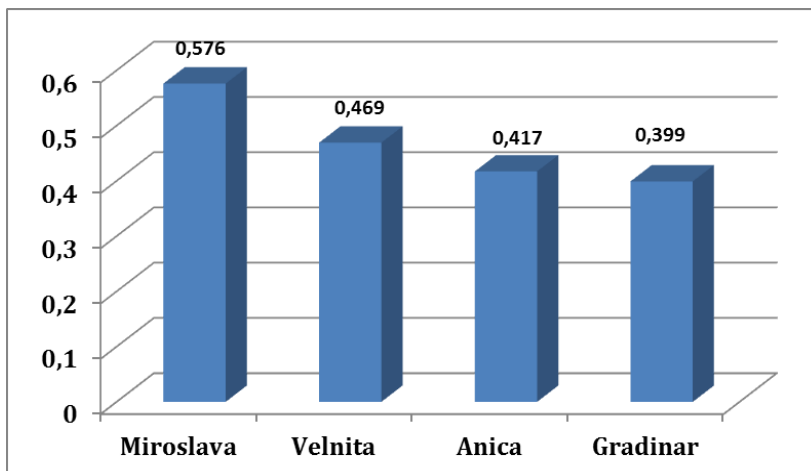


Fig. 4 Peroxidase activity in walnut leaves [UP / (g · min)]

The differences in the increase in the peroxidase activity can be considered a practical measure for assessing the level of stress, which causes an imbalance in the growth of the graft together with the rootstock and are generally considered a symptom of incompatibility (Tubbs, 1973). In the case of these walnut varieties, the increase in the peroxidase activity was correlated with the percentage of grafting success for the four walnut varieties.

The data from the literature shows that grafting may disrupt the circulation of nutrients in the tree as a whole, which influences the peroxidase activity in the leaves (Bower, 1981).

Thus, the results obtained for the four walnut varieties grafted on a rootstock represented by a local biotype reflect the influence of grafting on the level of peroxidase activity.

In the case of the varieties with a low percentage of grafting success (Mirosłava and Velnița) there was an increase in the peroxidase activity, while in the case of the varieties with a percentage of over 50% (Grădinari), the peroxidase activity was much lower.

The highest increases in the peroxidase activity were highlighted by the Mirosłava (the average content being of 0.576 UP / (g • min)) and Velnița (the average content being of 0.469 UP / (g • min)) varieties, and the lowest level of peroxidase activity was obtained for the Grădinari variety (the average content being of 0.399 UP / (g • min)).

CONCLUSIONS

1. The grafting of the four walnut varieties using the chip budding method determined a percentage of grafting success that varied from 47.5% for the Mirosłava variety to 55% for the Grădinari variety.

2. Of the four grafted walnut varieties, the highest growth vigor was presented by the Anica and Grădinari varieties, which also presented the highest percentage of grafting success, and trees with the highest height and diameter.

3. The ratios of chlorophyll a / b, chlorophyll / carotenoid pigments were within normal limits, which suggests that at the physiological level there are no changes determined by the grafted partners.

4. The highest increases in the peroxidase activity were highlighted by the Mirosłava variety, which had the lowest percentage of grafting success and the lowest level of peroxidase activity was obtained in the case of the Grădinari variety, which had the highest percentage of grafting success.

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