horticulturae

MDPI

Article

Evaluation of Walnut (*Juglans regia* L.) Grafting Performance by Optimizing Methods and Execution Periods Using TOPSIS Multicriteria Analysis

Cristina Zlati [1], Roxana Pașcu [1,*], Marius Florea [2], Marius Dascălu [1], Andromeda Pătrașcu Sonea [1] and Mihai Istrate [1]

[1] "Ion Ionescu de la Brad" Iasi University of Life Science, 3 Mihail Sadoveanu Alley, 700490 Iasi, Romania; cristina.zlati@iuls.ro (C.Z.); andromeda.patrascu@iuls.ro (A.P.S.)
[2] P.F.A. Florea Marius, 700490 Iasi, Romania
* Correspondence: roxana.pascu@iuls.ro

Abstract

Walnut multiplication technology for obtaining high-quality planting material consists of grafting, followed by forcing bionts in protected spaces under controlled microclimatic conditions, and completed by acclimatization under field conditions. The present research substantiates the hypothesis that the use of protected spaces (polyethylene tunnels) enables rigorous control of limiting factors. The main objectives of the paper are the comparative evaluation of two grafting methods (chip and patch budding) on the grafting success of eight native genotypes ('Anica', 'Grădinar', 'Miroslava', and 'Velnița', 'Bălțăți', 'Belcești', 'Săbăuani', and 'Șorogari') grafted on 'Bălțați' local biotype, determining the optimal moment of grafting by identifying the time window (April vs. August) that maximizes the success rate of the grafting association. The study, carried out from 2022 to 2024, evaluated the performance of chip and patch budding executed under high-tunnel conditions, quantifying the scion/rootstock growth, callus formation, and anatomical symbiont similarity through cross-sectional microscopy and image analysis software to measure vessel number, density, and diameter; the results are presented as the mean values of three annual repetitions across the experimental period. Preliminary results indicate a superior efficiency of the chip budding method, with a 51.3% success rate compared to 32.9% for the patch budding method. Another objective of the study was the ranking of the experimental variants. Thus, the Technique for Order of Preference by Similarity to Ideal Solution (TOPSIS), a multi-criteria decision analysis method, ranked the experimental variants and identified the chip budding performed in April (variant a1/b2) as the optimal solution across all analyzed physiological and morphological parameters. These findings are highly significant for the nursery sector, as they demonstrate that transitioning from unpredictable field conditions to controlled high-tunnel conditions stabilizes production outcomes. By establishing a clear methodological hierarchy and a precise chronological window, this study provides actionable guidelines to standardize walnut multiplication, mitigate seasonal climate risks, and substantially increase the output of high-quality certified planting material.

Keywords: grafting method; microscopic observations; *Juglans regia*; protected area; ranking; TOPSIS method

Check for updates

Academic Editor: Darius Kviklys

Received: 11 May 2026
Revised: 10 June 2026
Accepted: 11 June 2026
Published: 17 June 2026

1. Introduction

Walnut (*Juglans regia* L.), one of the oldest cultivated fruit species worldwide, is a strategic resource for human nutrition, recognized as an essential source of protein and bioactive compounds with proven health benefits [1,2]. Regarding multiplication technology, walnut presents increased difficulty in grafting compared to other fruit species, with grafting success limited by anatomical peculiarities, particularly the presence of extensive areas of non-generative medullary tissue in the branch structure. This intrinsic resistance necessitated in-depth research to optimize grafting techniques and forcing conditions [3–11].

The efficiency of histogenesis at the grafting point is conditioned by a complex interaction of factors: execution technique, mostly used is chip budding [12–14] or patch budding [15–17]; microclimatic parameters (temperature, humidity, light intensity); biochemical indicators (hormonal balance, profile of phenolic compounds) [3,4,16–23]; the degree of biont maturation [3,24,25] and, critically, the optimal timing of intervention [3,12,13,16,20,25]. According to specialized literature, the periods of maximum cambial activity (the beginning of April and the end of September) are the most favorable for the establishment of vascular connections.

Numerous studies have evaluated grafting performance in both controlled environments, where thermohydric parameters are maintained at optimal levels [3,12,15,26–30], and field conditions [31–35], and have also explored the role of growth regulators [36,37]. Additionally, the age of the rootstock directly influences the success rate; research indicates that rootstocks planted in vegetative rest and grafted by forcing in the same year can achieve a yield of about 78% [38].

From a physiological perspective, the correlation between vascular anatomy and the plant's water regime is essential. Histological studies indicate that reduced rootstock vigor is often associated with narrower or fewer xylem vessels in the grafting area, which limits the hydraulic conductivity between scion and rootstock [17,39]. At the same time, some research [40] approaches the distribution of phenotypes in the case of Mendelian hybrids.

The general objective of the research was to optimize the walnut grafting production process in protected areas. The specific objectives pursued were

- The comparative evaluation of the grafting methods, which was carried out by analyzing the efficiency of the chip and patch budding methods on the success of grafting of eight native genotypes ('Anica', 'Grădinar', 'Miroslava', 'Velnița', 'Bălțăți', 'Belcești', 'Săbăuani', and 'Șorogari') grafted on 'Bălțați' local biotype.
- Determining the optimal moment of grafting by identifying the time window (April vs. August) that maximizes the success rate of the grafting association.
- Histo-anatomical analysis of the grafting area that was achieved by monitoring callus formation and restoration of vascular continuity through microscopic observations.
- Hierarchy of experimental variants by using mathematical algorithms (CRITIC and TOPSIS) to select the best performing technological combination.

The present research also underpins an integrated analysis of advances in walnut grafting point formation, correlating early anatomical observations with technological success. Considering the multidimensionality of the factors involved, we used multicriteria decision analysis (MCDA) methods. The study uses the Criteria Importance Through Intercriteria Correlation (CRITIC) method, developed by Diakoulaki (1995) [41], for the objective determination of the weight of the experimental criteria, followed by the application of the Technique for Order of Preference by Similarity to Ideal Solution (TOPSIS) algorithm, based on Hwang and Yoon (1981) [42], for ranking the variants and identifying the best performing technological combination.

https://doi.org/10.3390/horticulturae12060742

2. Materials and Methods

2.1. Study Site and Technological Conditions

The nursery was established in a protected environment located in Belcești, Iași County, Romania (47.3 °N 27.08 °E) at an altitude of 90 m. The region has a temperate climate, with an average annual temperature of 9.8 °C (annual minimum = −18 °C, maximum = 36 °C), and the mean annual precipitation in the study area was 488 mm (recorded during the 2022–2024 period).

Cultivation was conducted within a polyethylene high tunnel measuring 20 m in length, 10 m in width, and 4.5 m in height at the center.

Harvested nuts intended for rootstock were treated with fungicides. Seeds were stratified in sand for eight weeks at a constant temperature of 4–5 °C. Sprouted walnuts were sown at a depth of 5 cm, with a spacing of 75 cm between rows and 10 cm between individual walnuts within each row.

Rootstock seedlings were grafted at two distinct intervals:

- summer grafting, performed in August when seedlings reached 7 months of age.
- spring grafting, performed the following April at 13 months of age.

For the April grafting, scion wood was harvested in late autumn and stored in a controlled warehouse at 2–4 °C over the winter to maintain dormancy.

The planting material used for the experiment was provided by P.F.A. Florea Marius farm, Bălțați, Iasi, Romania.

2.2. Biological Material and Grafting Methodology

The study utilized eight Romanian walnut (*Juglans regia* L.) genotypes as scions. The rootstock consisted of generative seedlings derived from the 'Bălțați' local biotype, sourced from Iași County. This cultivar was chosen as the universal rootstock for all experimental variants because of its exceptional local adaptability and vigor, which ensure uniformity of the planting material, and its specific agronomic advantages: high resistance to frost, common diseases, and pests. Fruit characteristics are: medium-sized nuts (10–12 g), elongated-oval in shape, with a well-filled kernel. Experimental factors are *Eight Genotypes*: 4 cultivars ('Anica', 'Velnița', 'Grădinar', and 'Miroslava'), and 4 local selections ('Bălțați', 'Belcești', 'Săbăuani', and 'Șorogari'). To maintain uniformity between established cultivars and local selections, the term 'genotype' was adopted as an umbrella designation throughout the study.

One Rootstock: 'Bălțați' local cultivar. Rootstock alone was used as the control.

Two Methods of grafting: a_1 = chip budding; a_2 = patch budding.

Two Time and phenological stage:

b_1 = August (dormant bud grafting, using fully matured vegetative buds collected from the current season's semi-lignified shoots).

b_2 = April (growing bud grafting, using dormant winter buds from the previous season's lignified shoots before bud break).

A total of 1600 seedlings were utilized (200 seedlings per variant). The experiment was repeated annually over the three consecutive years of the experiment (2022–2024). Data on compatibility (% survival), callus formation, and plant growth were analyzed using three-year averages, with each individual year serving as an experimental replication.

Grafting success was calculated as the percentage of total grafted plants that formed a successful structural union. Grafting success, was measured as a structural take, meaning, the scion bud physically fused with the rootstock, cambial bridge was formed, and the tissue remained alive (30 days post-grafting).

Bud break (buds started in vegetation), measures functional growth. It is the percentage of grafts in which the bud actually broke dormancy, pushed through, and produced vegetative shoot growth (measured in the following months). Bud-break rate was calculated as a conditional percentage, using only the successfully grafted plants. Thus, 'buds started in vegetation' is a subset restricted to surviving individuals.

To ensure high accuracy and conclusive results, the following parameters were monitored:

- Biometric determinations, measuring the physical growth and vigor of the grafted plants.
- Laboratory analyses, evaluating the physiological success of the graft union and the subsequent development of the genotypes.

2.3. Sample Preparation for Microscopic Observations

Sectioning of the grafting area was performed using the tissue preservation technique according to the method described by Santamarina Siurana et al., 2018 [43]. This procedure excluded the inclusion of biological material in resin or paraffin, thus facilitating the study and observation of cells and tissues without structural distortions or processing artifacts.

The samples consisted of stem segments measuring 5–6 cm, fixed in 70% ethyl alcohol. These were harvested from three critical points in the study area: above the grafting point, below the grafting point, and from the junction area (scion-rootstock interface), as shown in Figure 1.

Figure 1. The locations of the grafting area where the microsections were taken.

Harvesting was carried out at one and three months after grafting.

Because grafting success rates varied across combinations, we collected at least 10 independent biological replicates (individual grafted plants). From each graft union, 30 longitudinal/transverse sections were cut (10 above the grafting union, 10 in the grafting area, and 10 under the grafting union), and 10 representative fields of view were observed and imaged per section under the microscope.

Transverse and longitudinal sections were made using a Slee Mainz CUT 6062 microtome, with thicknesses ranging from 15 to 22 µm. Microscopic observations were performed using a MOTIC B series optical microscope (model 564–565), equipped with 4× and 10× objectives. To assess vascularization success, transverse sections were used to measure the radial and tangential diameters of the conducting vessels. Measurements were performed directly through the optical system using the MOTIC Image Plus 2.0 and ImageJ software 1.x.

2.4. Microscopic Observations and Histological Indicators

Microscopic analysis was performed on samples from the eight grafting combinations, with sections being collected from three distinct areas (Figure 1): above the grafting point (scion), from the junction area (scion-rootstock interface), and below the grafting point (rootstock).

Histological evaluation focused on the following parameters:

Vascular dynamics by monitoring the formation of new vascular tissues, vessel orientation, and cambial continuity between the scion and the rootstock.

Quantitative analysis of the xylem: vascular density (number of vessels per mm^2) and the size of the histological elements were determined by the average diameter of the xylem vessels, expressed in micrometers. We measured 40 individual vessels per section, across 3 sections per plant, using 5 biological replicate plants per graft combination, yielding a total of 600 vessel measurements per treatment. We have calculated the equivalent circular diameter from the cross-sectional area of each vessel lumen.

Tissue integrity was evaluated by the presence of necrotic layers at the grafting interface.

Meristematic activity was monitored by observing the new cambial tissue, and its ability to restore the vascular connection between the two partners was observed.

2.5. Statistical Analysis

Statistical data processing was performed using Duncan's post hoc test [44]. The statistical significance of the differences between means was reported at a probability threshold of $p \leq 0.05$. Calculations were performed using SPSS software v30 (IBM Corp., Armonk, NY, USA), and the interpretation of the results included calculating the limit differences LD (Limit Differences) according to the methodology described by Săulescu et al., 1967 [45].

2.6. Anatomical Similarity Index of the Symbionts (ASIS)

For the determination of the symbionts' anatomical resemblance index, measurements of xylem vessel number and diameter were made on sections taken from each of the scion-rootstock combinations that were studied. The index was calculated using the formula defined by the Research and Development Institute for Fruit Growing, Piteşti-Mărăcineni [17,46,47], where values close to 1.0 express good compatibility between the scion and rootstock, whereas values greater or less than 1.0 indicate poor compatibility.

$$\boldsymbol{ASIS} = \frac{\frac{\frac{Nr.v.s.}{Nr.v.r.} + \frac{Nr.v.s.}{Nr.v.g.} + \frac{Nr.v.r.}{Nr.v.g.}}{3}}{\frac{\frac{\varphi v.s.}{\varphi v.r.} + \frac{\varphi v.s.}{\varphi v.g.} + \frac{\varphi v.r.}{\varphi v.g.}}{3}}$$

where

Nr. v. s. = xylem vessels number at 10 cm above the joining area (scion);
Nr. v. r. = xylem vessels number at 10 cm under the joining area (rootstock);
Nr. v. g. = xylem vessels number in the grafting area;
φ v. s. = vessels average diameter at 10 cm above the joining area (scion);
φ v. r. = vessels average diameter at 10 cm under the joining area (rootstock);
φ v. g. = vessels average diameter in the grafting area.

2.7. Mathematical Analysis

The Criteria Importance Through Intercriteria Correlation (CRITIC) method is a technique used in multi-criteria decision-making (MCDM) problems, aimed at objectively determining the weights of each evaluation criterion. Unlike subjective methods, this approach extracts the importance of the criteria directly from the data structure, taking into account both the intensity contrast (standard deviation) and the conflict between the criteria (correlation). The CRITIC algorithm involves the following steps:

1. The decision matrix is considered.
2. The decision matrix is normalized.
3. The standard deviation is calculated for each individual criterion σ_i.
4. The correlation matrix is written, formed by calculating the Pearson correlation coefficient between the criteria (r_{ik}).

5. For each criterion i, it is calculated $C_i = \sigma_i \times \sum_{k=1}^{n}(1 - r_{ik})$, where the sum $\sum_{k=1}^{n}(1 - r_{ik})$ represents the level of non-correlation between the criteria.
6. The shares are normalized, thus $w_i = \frac{C_i}{\sum_{i=1}^{n} C_i}$.

Within this study, the evaluation criteria comprise: grafting success (%), callus formation scale from 0 (no compatibility) to 4 (high compatibility), scion height (cm), scion diameter (cm), rootstock diameter (cm), and ASIS.

The decision matrix consists of the average values obtained for each individual experiment. The obtained shares are necessary to establish the hierarchy of the experimental variants. In this case, we propose applying the TOPSIS method.

The **TOPSIS** method is a ranking method, structured on six steps. We consider an MCDM problem with m alternatives and n criteria. Using the TOPSIS method involves the following steps:

1. A decision matrix is created, D = (x_{mn}), where x_{mn} represents the influence of n criterion, upon the m alternative.
2. The decision matrix is normalized as follows:

$$r_{ij} = \frac{x_{ij}}{\sqrt{\sum_{i=1}^{m} x_{ij}^2}},\ i \in \{1,\ 2,\ \ldots,\ m\},\ j \in \{1,\ 2, \ldots,\ n\}.$$

3. Multiply the columns of the normalized decision matrix with the associated shares to obtain the shared normalized decision matrix.

$$A = (v_{mn});\ v_{ij} = w_j \times r_{ij},$$

where w_j, $j \in \{1,\ 2,\ \ldots,\ n\}$ represents the share of j criterion.

4. Determine the positive ideal solution and the negative ideal solution, thus:

$$A^+ = \{v_1^+,\ v_2^+,\ \ldots,\ v_n^+\} = \{(max\ v_{ij}/\ j \in K_b)(min\ v_{ij}\ /\ j \in K_c),\ i \in \{1,\ 2,\ \ldots,\ m\}\}$$

$$A^- = \{v_1^-,\ v_2^-, \ldots,\ v_n^-\} = \{(min\ v_{ij}/\ j \in K_b)(max\ v_{ij}\ /\ j \in K_c),\ i \in \{1,\ 2, \ldots,\ m\}\}$$

where K_b represents the set of criteria that refers to benefits and Kc represents the set of criteria that refers to costs.

5. Measures are obtained to separate the existing alternatives, compared to the positive ideal solution and the negative ideal solution. Denote by S_i^+ the separation is measured based on the Euclidean distance between each alternative and the positive ideal solution. Similarly, S_I^-, $i \in \{1,\ 2, \ldots,\ m\}$ is defined. Therefore,

$$S_i^+ = \sqrt{\sum_{j=1}^{n} \left(v_j^+ - v_{ij}\right)^2},\ i \in \{1,2,\ \ldots,\ m\},\ j \in \{1,\ 2,\ \ldots,\ n\},$$

$$S_i^- = \sqrt{\sum_{j=1}^{n} \left(v_j^- - v_{ij}\right)^2},\ i \in \{1,2,\ \ldots,\ m\},\ j \in \{1,\ 2,\ \ldots,\ n\}$$

6. The relative closeness to the ideal solution is calculated:

$$C_i = \frac{S_i^-}{S_i^- + S_i^+},\ i \in \{1,\ 2, \ldots,\ m\}.$$

The classification of the analyzed variants is determined by the descending order of these coefficients, the maximum value indicating the priority alternative. Due to its operational simplicity, computational efficiency, and rigorous mathematical foundation, the method has been widely adopted in various research fields [48–50].

3. Results

To comprehensively evaluate grafting compatibility and plant vigor, the results are presented across three distinct analytical stages: the anatomical and morphological development at the graft interface (including xylem vessel dynamics), the subsequent biometric growth and success rates, and finally, the multi-criteria ranking of the experimental variants based on grafting method and timing.

3.1. Environmental Conditions Influence on Grafting Success

In walnut propagation, environmental conditions, particularly temperature, are the most critical factors influencing graft union success. As cited in the specialized literature [12,15], the optimal range for callus formation is most active between 25 and 28 °C. The physiological process of callusing slows significantly outside this range and is completely inhibited at temperatures below 0 °C or above 38 °C.

The environmental conditions recorded during the 25 days post-grafting period are summarized in Table 1. April grafting yields significantly higher success rates than August grafting, regardless of the budding method used. April average (23.3 °C) is close to the optimal 25–28 °C range required for active callusing. Heat in August (with an average of 32.33 °C) correlates with a sharp decline in grafting success rates. Chip budding in April maintains the most stable and reliable success rate across all three years, consistently hovering between 50.1% and 52.6%. These data suggest that lower minimum temperatures in April are far less detrimental to graft success than the high temperatures of August.

Table 1. Climatic conditions 25 days post grafting, in high tunnel conditions, Belcești, Iași County.

Temperature Conditions		Years 2022	2023	2024	Average (2022–2024)
			April grafting		
Daily average temperatures (°C)		26.2	23.8	19.9	23.30
Air relative humidity (%)		61	57	68	62
Grafting success (%)	Chip budding	50.1	51.3	52.6	51.3
	Patch budding	31.3	33.8	33.6	32.9
			August grafting		
Daily average temperatures (°C)		36.5	31.2	29.3	32.33
Air relative humidity (%)		48	61	59	56
Grafting success (%)	Chip budding	20.1	20.3	18.7	19.7
	Patch budding	22.4	19.5	20.6	20.8

3.2. Anatomical and Morphological Changes That Occur in the Grafting Area 15- and 30-Days Post-Grafting, Using the Chip Budding Method

The success of grafting depends on a series of highly coordinated anatomical and morphological transitions at the interface of the scion and rootstock. Anatomical and morphological evaluations served as a primary step in determining the degree of compatibility between the scion and the 'Bălțați' rootstock. Success in walnut grafting depends on structural changes at the union site, including vessel orientation, fascicular continuity, and the presence or absence of lacunar areas.

Microscopic observations (Figure 2) of cross-sections taken during the early stages of scion bud development (15 days post-grafting) revealed structural discontinuity. Initial sections showed incomplete connections between parenchyma cells. Newly formed xylem vessels exhibited a nearly horizontal orientation, deviating from their standard vertical alignment.

This phenomenon aligns with observations in other heterograft combinations, such as those documented in sweet cherry and plum cultivars [47,51]. We hypothesize that the high temperature fluctuations within the polyethylene high tunnel delayed the onset of callusing.

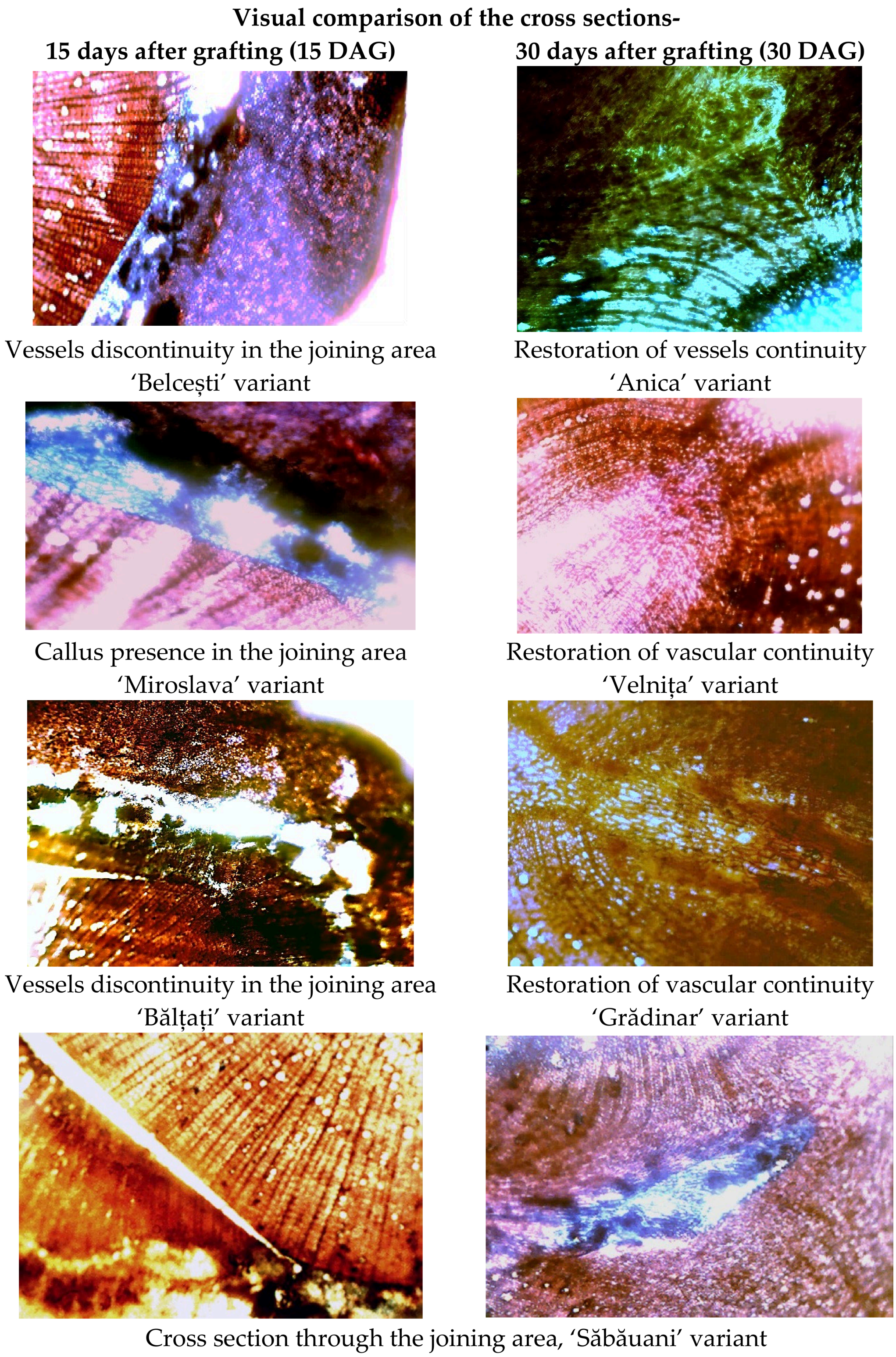

Figure 2. Cross-sections through the grafting area performed 15- and 30-days post grafting, using chip budding method, morphological development of studied genotypes grafted on *Juglans regia* rootstock—'Bălțați' local biotype. At 15 DAG, note the discontinuities and initial callus proliferation at the graft union interface. By 30 DAG, successful vascular differentiation is visible, indicating a good compatibility of the graft union.

Thirty days after grafting, particularly in the chip budding variants, microscopic analysis confirmed the establishment of vascular continuity. Cambial cells successfully differentiated from the callus, forming functional xylem and phloem elements. This continuity ensures the efficient bidirectional transport of water and nutrients, indicating a high level of compatibility between the local Romanian genotypes and the 'Bălțați' rootstock. No significant differences were observed among the eight genotypes regarding the timing of callus appearance or the formation of the vascular cambium.

Data regarding the newly formed vascular elements are summarized in Tables 2 and 3. While differences between genotypes were not statistically significant, they provide insight into early-stage transport efficiency. The number, diameter, and trajectory of woody vessels in the grafting area, varied depending on the cultivar and correlated with the relative success of the symbiont union. 'Anica', 'Miroslava', and 'Velnița' demonstrated the highest number of vessels with strong symmetry between scion and rootstock, exhibiting a trend toward high vessel density.

Table 2. Xylem vessels number in three analyzed areas.

Variety	Vessels Number Under the Grafting Area	Difference to the Control ±	Vessels Number in the Joining Area	Difference to the Control ±	Vessels Number Above the Grafting Area	Difference to the Control ±
'Anica'	53.20	4.35	48.10	2.35	55.60	2.65
'Bălțați'	43.90	−4.95	41.70	−4.05	50.20	−2.75
'Belcești'	50.10	1.25	47.40	1.65	49.30	−3.65
'Grădinar'	49.20	0.35	46.80	1.05	54.70	1.75
'Miroslava'	52.17	3.32	50.10	4.35	52.30	−0.65
'Săbăuani'	48.30	−0.55	45.70	−0.05	49.10	−3.85
'Șorogari'	47.72	−1.13	48.30	2.55	50.21	−2.74
'Velnița'	51.60	2.75	49.16	3.41	52.80	−0.15
Rootstock—the control	48.85	-	45.75	-	52.95	-
	LD 5% = 4.68 LD 1% = 6.60 LD 0.1% = 10.82 vessels		LD 5% = 4.53 LD 1% = 6.86 LD 0.1% = 11.02 vessels		LD 5% = 4.60 LD 1% = 6.97 LD 0.1% = 11.20 vessels	

LD ($p < 0.05$) = Limit Differences for significance threshold.

Table 3. Xylem vessels diameter (micrometer—μ) in three analyzed areas.

Cultivar	Vessels Diameter Under the Grafting Area	Difference to the Control (μm)	Vessels Diameter in the Joining Area	Difference to the Control (μ)	Vessels Diameter Above the Grafting Area	Difference to the Control (μ)
'Anica'	4.45	0.22	4.71	0.11	4.43	−0.42
'Bălțați'	4.02	−0.21	4.24	−0.36	4.61	0.24
'Belcești'	4.11	−0.12	4.23	−0.37	4.77	−0.08
'Grădinar'	4.51	0.28	4.30	−0.30	5.01	0.16
'Miroslava'	4.20	−0.03	4.27	−0.33	4.64	−0.21
'Săbăuani'	4.62	0.39	4.48	−0.12	4.90	0.05
'Șorogari'	4.86	−0.63	4.21	−0.39	4.42	0.12
'Velnița'	3.99	−0.24	4.58	−0.02	4.88	0.03
Rootstock—the control	4.23	-	4.60	-	4.85	-
	LD 5% = 0.61 LD 1% = 0.92 LD 0.1% = 1.49 μ		LD 5% = 0.26 LD 1% = 0.40 LD 0.1% = 0.65 μ		LD 5% = 0.24 LD 1% = 0.37 LD 0.1% = 0.59 μ	

LD ($p < 0.05$) = Limit Differences for significance threshold.

In terms of vessel diameter analysis, 'Velnița' recorded the smallest vessel diameter below the graft union (3.99 μ). 'Șorogari' exhibited the smallest diameters within the union (4.21 μ) and above it (4.42 μ). The variations in vessel diameter and frequency between the scion and rootstock may explain the localized transport resistance observed during the initial stages of wound healing.

Data on vascular elements helped us to determine the anatomical similarity index of the symbionts. The results drove us to a series of indexes that allowed us to appreciate the varieties compatibility scale with the rootstock. An index value close to 1.0 indicates good compatibility (such as 1.07 for 'Anica', 0.96 for 'Velnița', 1.02 for 'Miroslava' and 0.95 for 'Grădinar'), while an index which deviates from 1.0, such as 'Săbăuani' (1.2), 'Bălțați' (1.28) 'Belcești' (1.29) and 'Șorogari' (1.31), indicates medium or late compatibility (Figure 3).

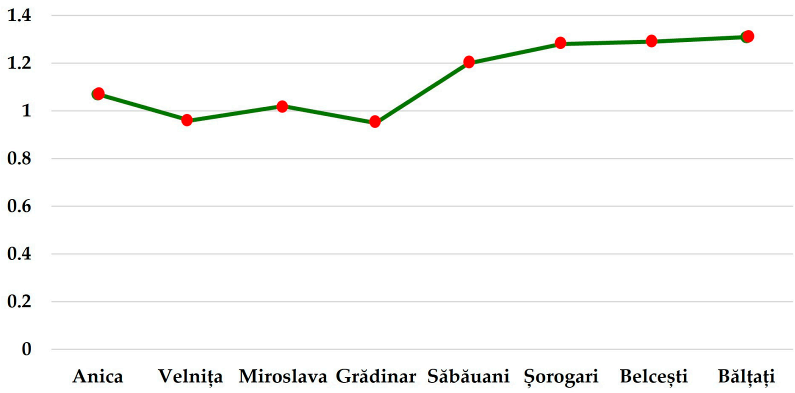

Figure 3. The anatomical similarity index of the symbionts of the eight walnut genotypes grafted onto the rootstock *Juglans regia*, chip budding method grafted in April variant (a1/b2): Anica-1.07, Velnița-0.96, Miroslava-1.02, Grădinar-0.95, Săbăuani-1.2, Șorogari-1.31, Belcești-1.29 and Bălțați-1.28.

3.3. Influence of Grafting Methods and Time on Callus Formation, Grafting Success, and Plant Growth in Experimental Conditions

The experimental variants discussed throughout this section are defined by their specific treatment combinations: grafting methods a1 (chip budding) and a2 (patch budding), and execution windows b1 (August) and b2 (April).

According to the data in Table 4, the chip budding method performed in April (variant a1/b2) achieved the highest success rate at 51.3%. During the same period, the patch method (variant a2/b2) resulted in a 32.9% success rate. In contrast, grafting performed in August (b1) showed a marked decline in viability, registering a success rate ranging from 19.7% to 20.8%.

Table 4. Grafting method influence on grafting success and callus formation in protected space (2022–2024).

Experimental Variants	Grafting Success (%)	Callus Formation Scale (0–4)	ASIS
Chip budding in August (a_1/b_1)			
'Anica'_a_1/b_1	20.7	3.9	1.09
'Bălțați'_a_1/b_1	22.6	4.0	1.51
'Belcești'_a_1/b_1	20.3	3.8	1.42
'Grădinar'_a_1/b_1	18.7	3.7	0.92
'Miroslava'_a_1/b_1	17.5	3.8	1.08
'Săbăuani'_a_1/b_1	19.8	3.5	1.17
'Șorogari'_a_1/b_1	17.3	3.9	1.33
'Velnița'_a_1/b_1	20.7	4.0	1.08
Average_a_1/b_1	19.7 c *	3.8 a *	1.20 a *

Table 4. *Cont.*

Experimental Variants	Grafting Success (%)	Callus Formation Scale (0–4)	ASIS
Chip budding in April (a_1/b_2)			
'Anica'_a_1/b_2	52.3	3.6	1.07
'Bălțați'_a_1/b_2	49.8	3.7	1.28
'Belcești'_a_1/b_2	52.6	3.9	1.29
'Grădinar'_a_1/b_2	49.3	3.7	0.95
'Miroslava'_a_1/b_2	51.5	3.8	1.02
'Săbăuani'_a_1/b_2	51.6	3.5	1.26
'Șorogari'_a_1/b_2	49.8	3.6	1.31
'Velnița'_a_1/b_2	53.5	3.8	0.96
Average_a_1/b_2	51.3 a *	3.7 a *	1.14 a *
Patch budding in August (a_2/b_1)			
'Anica'_a_2/b_1	21.8	3.4	1.10
'Bălțați'_a_2/b_1	20.5	3.1	1.47
'Belcești'_a_2/b_1	22.1	3.3	1.51
'Grădinar'_a_2/b_1	18.7	3.6	0.97
'Miroslava'_a_2/b_1	20.4	3.4	1.06
'Săbăuani'_a_2/b_1	18.2	3.5	1.24
'Șorogari'_a_2/b_1	21.9	3.4	1.29
'Velnița'_a_2/b_1	22.8	3.5	1.12
Average_a_2/b_1	20.8 c *	3.4 a *	1.22 a *
Patch budding in April (a_2/b_2)			
'Anica'_a_2/b_2	31.8	3.8	1.08
'Bălțați'_a_2/b_2	33.6	3.9	1.46
'Belcești'_a_2/b_2	33.3	3.4	1.39
'Grădinar'_a_2/b_2	33.7	3.8	1.04
'Miroslava'_a_2/b_2	30.8	3.5	1.05
'Săbăuani'_a_2/b_2	33.4	3.3	1.25
'Șorogari'_a_2/b_2	32.6	3.6	1.24
'Velnița'_a_2/b_2	34.0	3.5	1.17
Average_a_2/b_2	32.9 b *	3.6 a *	1.21 a *

A—method of grafting: a_1 = chip budding; a_2 = patch budding; B—time of grafting: b_1 = August (dormant bud); b_2 = April (growing bud); * Duncan test, $p < 0.05$. Different letters between treatment combinations (grafting method x time) denote significant differences.

Callus formation was assessed visually approximately 30 days after grafting, using a scale from 1 to 4. An essential condition for callus formation is the perfect overlapping of the cambial areas of both partners across the largest possible contact surface.

Based on these evaluations, callus formation, assessed 30 days post-grafting, ranged between 3.1 and 4.0, indicating a generally high level of compatibility. This success is attributed to the precise overlapping of cambial areas between the rootstock and scion. However, the chip budding method (a1) consistently outperformed the other variants. Regarding chip budding, the grafting period had a pronounced effect on success rates. Specifically, grafting in April resulted in a significantly higher graft success rate (51.3%) compared to August (19.7%), as detailed in Table 4. The success of walnut grafting depends critically on matching the chosen method with the optimal execution period.

The present research shows that the chip budding method (a1) is superior, offering not only the highest grafting success rate but also the best callus quality and growth, regardless of the season. Although a success rate over 30% is considered a good result for this species, the use of the chip budding method ensures optimal tissue healing (score of 3.7–3.8) and vigorous graft development, making it the recommended optimal solution for walnut propagation.

The data in Table 5 show that the grafting method and timing significantly influence scion development across all measured parameters.

Table 5. Interaction of grafting methods and grafting period on scion growth (2022–2024).

Experimental Variants	Graft Buds Started in Vegetation (%)	Scion Height (cm)	Scion Diameter (cm)	Rootstock Diameter (cm)	Number of Leaflets/Plant
Chip budding in August (a_1/b_1)					
'Anica'_a_1/b_1	20.1	160.3	2.16	2.48	148.20
'Bălțați'_a_1/b_1	19.4	161.4	2.17	2.45	146.21
'Belcești'_a_1/b_1	18.1	158.9	2.15	2.44	142.35
'Grădinar'_a_1/b_1	17.1	156.8	2.13	2.51	135.12
'Miroslava'_a_1/b_1	18.5	157.6	2.14	2.36	143.43
'Săbăuani'_a_1/b_1	18.2	158.2	2.15	2.38	147.42
'Șorogari'_a_1/b_1	17.3	159.7	2.13	2.33	144.38
'Velnița'_a_1/b_1	17.7	157.5	2.17	2.41	139.85
Average_a_1/b_1	18.3 a *	158.8 a *	2.15 a *	2.42 a *	143.37 a *
Chip budding in April (a_1/b_2)					
'Anica'_a_1/b_2	48.6	166.5	2.43	2.86	135.72
'Bălțați'_a_1/b_2	48.3	165.9	2.41	3.10	134.61
'Belcești'_a_1/b_2	49.3	168.3	2.35	2.90	134.99
'Grădinar'_a_1/b_2	46.1	167.4	2.37	2.83	133.64
'Miroslava'_a_1/b_2	47.3	169.2	2.46	2.75	135.11
'Săbăuani'_a_1/b_2	48.6	167.8	2.44	2.91	134.48
'Șorogari'_a_1/b_2	49.0	168.3	2.41	2.68	132.98
'Velnița'_a_1/b_2	45.2	169.8	2.49	2.77	133.91
Average_a_1/b_2	47.8 c *	167.9 b *	2.42 b *	2.85 c *	134.43 a *
Patch budding in August (a_2/b_1)					
'Anica'_a_2/b_1	30.2	132.1	2.17	2.56	130.86
'Bălțați'_a_2/b_1	29.5	132.6	2.11	2.51	130.35
'Belcești'_a_2/b_1	28.1	132.1	2.10	2.61	133.17
'Grădinar'_a_2/b_1	29.8	129.2	2.10	2.58	131.20
'Miroslava'_a_2/b_1	28.6	132.7	2.18	2.47	124.52
'Săbăuani'_a_2/b_1	29.3	128.6	2.17	2.59	129.23
'Șorogari'_a_2/b_1	26.5	131.3	2.16	2.48	132.72
'Velnița'_a_2/b_1	27.6	129.4	2.21	2.52	128.91
Average_a_2/b_1	28.7 b *	131.0 c *	2.15 a *	2.54 b *	130.12 a *
Patch budding in April (a_2/b_2)					
'Anica'_a_2/b_2	17.3	161.3	2.20	2.68	142.31
'Bălțați'_a_2/b_2	17.4	159.3	2.1	2.57	142.68
'Belcești'_a_2/b_2	18.3	158.0	2.22	2.71	141.87
'Grădinar'_a_2/b_2	17.6	160.2	2.24	2.72	139.46
'Miroslava'_a_2/b_2	17.8	158.5	2.27	2.63	142.28
'Săbăuani'_a_2/b_2	19.3	157.2	2.22	2.7	143.91
'Șorogari'_a_2/b_2	18.2	161.4	2.25	2.68	141.82
'Velnița'_a_2/b_2	19.7	160.1	2.26	2.59	140.63
Average_a_2/b_2	18.2 a *	159.5 a *	2.21 a *	2.66 c *	141.87 a *

A—method of grafting: a_1 = chip budding; a_2 = patch budding; B—time of grafting: b_1 = August (dormant bud); b_2 = April (growing bud); * Duncan test, $p < 0.05$. Different letters between treatment combinations (grafting method x time) denote significant differences.

The chip budding method in April (a1/b2) achieved the highest success rate, with an average of 47.8% of buds entering the vegetative stage in the following spring. In contrast, success rates for dormant buds in August (a1/b1) and the patch method in April (a2/b2) were significantly lower, averaging approximately 18.2% and 18.3%, respectively.

Morphological growth was most pronounced in the a1/b2 variant, which produced the tallest scions (167.9 cm). Conversely, the shortest scions were recorded in the August patch budding (a2/b1) variant, averaging only 131.0 cm.

Stem diameter measurements (for both scion and rootstock diameter) further confirm the superiority of the April chip budding method. With values of 2.42 cm and 2.85 cm, respectively, this variant exhibited more robust structural development compared to all other experimental groups.

Interestingly, although the August chip budding (a1/b1) had a lower overall success rate, the surviving plants produced the highest number of leaflets (143.37), closely followed by the April patch budding (a2/b2) at 141.87. This suggests that while fewer grafts take in these variants, the resulting plants develop dense foliage.

Based on the analysis of the data in Table 5, it is confirmed that the a1/b2 variant (chip budding performed in April) is the most efficient method for walnut grafting. It recorded not only the highest vegetative initiation rate (47.8%), but also the most vigorous growth in height (167.9 cm) and diameter. Although other variants showed good leaf density, the overall development of the scion is clearly superior in the case of spring chip budding. Therefore, to maximize both the success rate and the vigor of the planting material, using the chip budding method in April is recommended. This approach optimizes walnut seedling development across all genotypes, resulting in significantly improved bud burst, superior structural diameters, and a well-balanced canopy.

3.4. Ranking the Variants Using the TOPSIS Method

To evaluate the experimental variants, the criteria selected include grafting success (%), callus formation scale (0–4), scion height (cm), scion diameter (cm), rootstock diameter (cm), and ASIS. The decision matrix consists of the average values obtained for each experimental variant over the three years of the study. The resulting rankings, derived from applying these methods, are presented in Table 6.

Table 6. The share of each individual criterion.

Variant	Grafting Success (%)	Callus Formation Scale (0–4)	Scion Height (cm)	Scion Diameter (cm)	Rootstock Diameter (cm)	ASIS
Chip budding/August (a_1/b_1)	12%	14%	14%	15%	29%	16%
Chip budding/April (a_1/b_2)	14%	15%	15%	22%	20%	15%
Patch budding/August (a_2/b_1)	15%	17%	13%	19%	14%	23%
Patch budding/April (a_2/b_2)	20%	18%	15%	18%	15%	15%

A—method of grafting: a_1 = chip budding; a_2 = patch budding; B—time of grafting: b_1 = August (dormant bud); b_2 = April (growing bud).

To determine the weights of each criterion, the CRITIC method was applied. As is widely recognized, when the ASIS index is closest to 1.0, it indicates a high degree of compatibility. For the criteria presented above, the maximum value is considered the best, and the minimum the worst. In order to analyze this aspect in the case of the ASIS coefficient as well, we propose a recalculation of it using the formula 1-absolute value∣1-ASIS COEFFICIENT∣. Therefore, the highest value of the new ASIS is best, and the lowest is weakest.

The CRITIC method was used to quantify the relative weight of each factor in differentiating the studied varieties. By evaluating both the contrast intensity and the correlation between criteria, this method identifies which parameters most significantly influence the final rankings. Therefore, the results of this analysis across all four experimental variants (a1/b1, a1/b2, a2/b1, a2/b2) are detailed in Table 6, providing a statistical basis for the importance assigned to each physiological and technological factor.

According to Table 6, the CRITIC weights exhibit a balanced distribution, with values ranging from 12% to 29%. This homogeneity suggests that the process of selecting the best scion-rootstock combinations is multifactorial.

An important aspect is the rootstock diameter for August grafting (b1), which yielded the highest weight (29%). This indicates that, in summer grafting, the rootstock's capacity to support development is the primary differentiating criterion. In contrast, in the a2/b1 variant, the ASIS index (23%) becomes the main indicator, demonstrating that the patch budding method is highly sensitive to the anatomical affinity between the partners.

The performance of the genotypes under each grafting method was analyzed for both April and August, using the TOPSIS method. In applying the TOPSIS method to our context, where the genotypes represent the alternatives and the parameters from Table 6 serve as the criteria, all selected metrics function as benefit criteria, with no cost criteria identified. The resulting proximity coefficients and final rankings are summarized in Table 7.

Table 7. Ranking the variants.

Nr. Crt.	Experiments Variants	Coefficient	Ranking
	Chip budding in August (a_1/b_1)		
1	'Anica' a1/b1	0.924	I
2	'Velnița' a1/b1	0.921	II
3	'Grădinar' a1/b1	0.855	III
4	'Miroslava' a1/b1	0.824	IV
5	'Săbăuani' a1/b1	0.748	V
6	'Șorogari' a1/b1	0.409	VI
7	'Belcești' a1/b1	0.241	VII
8	'Bălțați' a1/b1	0.196	VIII
	Chip budding in April (a_1/b_2)		
1	'Velnița' a1/b2	0.719	I
2	'Miroslava' a1/b2	0.717	II
3	'Grădinar' a1/b2	0.699	III
4	'Anica' a1/b2	0.698	IV
5	'Bălțați' a1/b2	0.352	V
6	'Belcești' a1/b2	0.279	VI
7	'Săbăuani' a1/b2	0.273	VII
8	'Șorogari' a1/b2	0.072	VIII
	Patch budding in August (a_2/b_1)		
1	'Anica' a2/b1	0.814	I
2	'Velnița' a2/b1	0.808	II
3	'Miroslava' a2/b1	0.781	III
4	''Grădinar' a2/b1	0.742	IV
5	Săbăuani' a2/b1	0.510	V
6	'Șorogari'' a2/b1	0.506	VI
7	'Belcești' a2/b1	0.272	VII
8	'Bălțați' a2/b1	0.189	VIII
	Patch budding in April (a_2/b_2)		
1	'Grădinar' a2/b2	0.936	I
2	'Anica' a2/b2	0.818	II
3	'Miroslava' a2/b2	0.731	III
4	'Velnița' a2/b2	0.645	IV
5	'Șorogari' a2/b2	0.532	V
6	'Săbăuani' a2/b2	0.469	VI
7	'Bălțați' a2/b2	0.301	VII
8	'Belcești' a2/b2	0.256	VIII

The TOPSIS ranking results (Table 7) reveal a clear distinction in how different walnut genotypes respond to grafting conditions.

In the overall ranking, the top four positions were consistently occupied by the same genotypes, 'Anica', 'Velnița', 'Grădinar', and 'Miroslava', although their exact hierarchy varied slightly across grafting methods and time periods. In contrast, the remaining cultivars ('Șorogari', 'Săbăuani', 'Bălțăți', and 'Belcești') exhibited significant variability in the TOPSIS rankings. Their performance appears highly dependent on the interaction between specific technological factors and the grafting period.

For a general conclusion, it is necessary to aggregate the performance of the genotypes across all methods and execution times. In this context, the overall weights of the criteria, established using the CRITICAL method, are presented in Table 8.

Table 8. The share of each individual criterion corresponding to all data.

Grafting Success (%)	Callus Formation Scale (0–4)	Scion Heigh (cm)	Scion Diameter (cm)	Rootstock Diameter (cm)	ASIS
15%	19%	17%	13%	12%	25%

The application of the TOPSIS method aims to establish a definitive hierarchy across all experiment variants. The results shown in Figure 4 represent the top 10-performing variants, each achieving a closeness coefficient greater than 0.6; all remaining experimental variants yielded coefficients below this threshold.

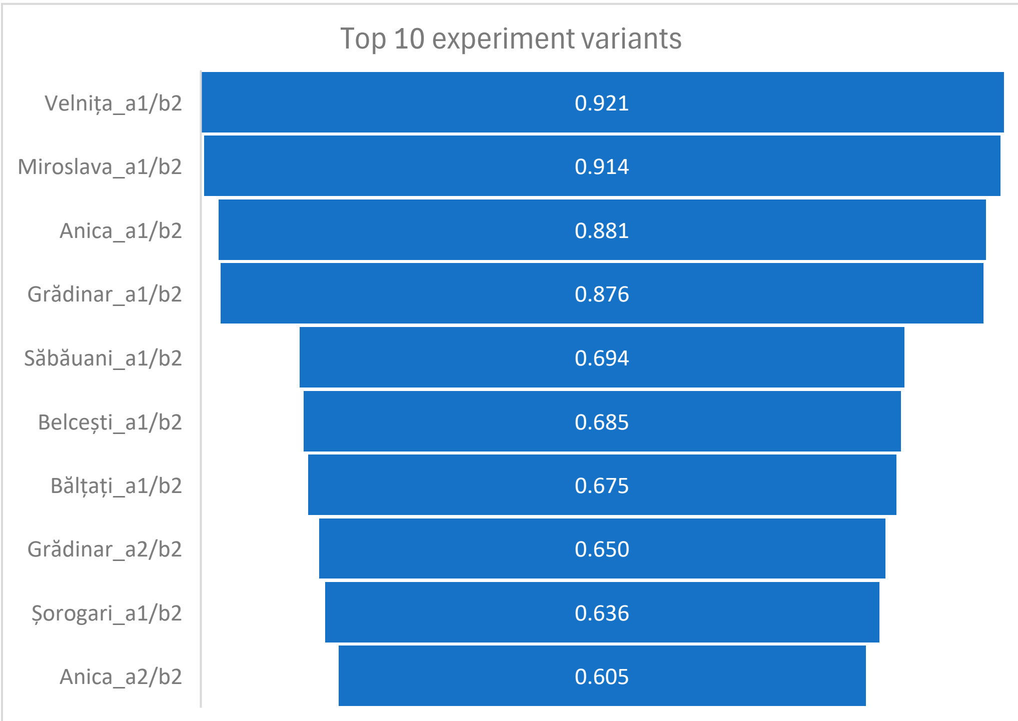

Figure 4. Ranking TOPSIS for all experiment variants.

Notably, the top seven positions are occupied from a1/b2 variant, which confirms that chip budding in April is the most highly recommended approach. Furthermore, the genotypes 'Velnița', 'Miroslava', 'Anica', and 'Grădinar' occupy the first four places within this ranking.

To better visualize the superior performance and balanced physiological profiles of the top four varieties, a radar diagram (Figure 5) was developed. This illustrates how these leading genotypes maintain high values across multiple metrics simultaneously, distinguishing them as the most reliable choices for grafting success. For this analysis, the values for each criterion were normalized, and the average value across all grafting methods and execution windows was calculated; the resulting scores are compiled in Table 9.

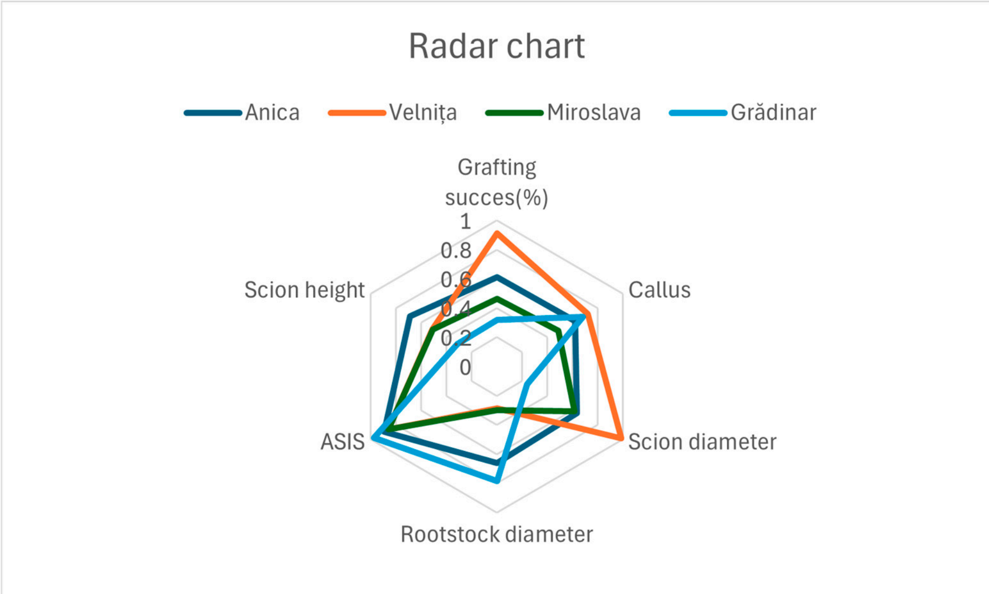

Figure 5. Radar diagram for top genotypes.

Table 9. Average normalized biometric indices of the first four varieties.

Variant	Grafting Success (%)	Callus Formation Scale (0–4)	Scion Heigh (cm)	Scion Diameter (cm)	Rootstock Diameter (cm)	ASIS
'Anica'	0.61	0.62	0.69	0.64	0.66	0.89
'Velnița'	0.91	0.72	0.51	0.99	0.29	0.86
'Miroslava'	0.46	0.49	0.51	0.62	0.30	0.86
'Grădinar'	0.32	0.68	0.31	0.24	0.79	0.97

The radar chart presented in Figure 5 illustrates the normalized data summarized in Table 9.

Figure 5 shows us that the 'Velnița' genotype exhibits the best overall performance, with its associated orange line enclosing the largest surface area on the radar chart. Its primary strengths are scion diameter and grafting success, which ensure the development of a robust structural union. The 'Grădinar' genotype demonstrates superior performance in the ASIS score, along with a high rootstock diameter. This indicates that 'Grădinar' is characterized by long-term graft longevity due to excellent vascular continuity at the union site. Meanwhile, the 'Anica' and 'Miroslava' genotypes exhibit consistent performance and similar profiles, making them highly reliable variants.

In summary, while spring chip budding (April) clearly optimizes overall grafting success, the distinct performance rankings among scion genotypes (Figure 5) indicate that the scion genotype is an equally critical factor in determining grafting compatibility and efficiency.

4. Discussion

Grafting conditions play a crucial role in histogenesis at the grafting interface and are governed by a complex interaction of interrelated factors, including execution technique, microclimatic parameters, biochemical indicators, plant maturation, and the optimal timing of intervention [40,52–55]. For these reasons, walnut grafting remains a critical and active area of research. Consequently, optimizing walnut grafting by evaluating two distinct techniques across two execution windows within a protected space (polyethylene high tunnel) represents a significant advancement in the development of nursery technology. The results of this study underscore the critical synergy between microclimatic control, execution timing, and anatomical compatibility. Given that grafting conditions are inherently variable, a multi-criteria decision analysis was used to interpret and better understand these complex interactions [56,57].

Performing grafting operations within polyethylene high tunnels successfully mitigated the fluctuating environmental conditions typical of open-field grafting in Romania, where success rates frequently suffer due to thermal instability. In this study, April grafting yielded the most favorable results compared to August interventions. This trend is supported by recent literature [58], which indicates that spring environmental conditions, specifically temperatures near 27 °C and relative humidity levels between 40% and 70%, are optimal for callus proliferation in *Juglans regia* L. The higher success rate of chip budding (51.3%) than patch budding in our protected environment suggests that the chip budding method provides more resilient cambial contact during early-spring phenological stages. This occurs despite some field studies conducted in alternative climates that favor patch budding due to its larger physical contact area [57,59].

Previous research has established that cambial cells successfully differentiate from the callus matrix to form functional xylem and phloem elements during the later stages of scion shoot development [60]. In this study, microscopic analysis confirmed the establishment of vascular continuity, particularly in the chip-budding variants. The minor variations observed in vessel diameter and frequency between the scion and rootstock may explain the localized transport resistance detected during the initial stages of graft establishment [58,61].

Furthermore, the interaction between grafting methods and execution timing significantly influences the ultimate survival rates of grafted plants [51,62–68]. Our results indicate that the chip budding method performed in April (variant a1/b2) achieved the highest success rate, followed by the patch method during the same period (variant a2/b2), as shown in Table 4. In contrast, grafting performed in August (b1), using dormant buds, exhibited a marked decline in viability, ranging from 19.7% to 20.8%. The study demonstrates that timing is a critical determinant factor of walnut grafting success. While both methods executed in April exceeded the 30% grafting success threshold recognized by researchers [64,69] as a commercially viable outcome for walnut propagation, spring chip budding proved to be the most effective approach. Conversely, August grafting with dormant buds proved insufficient for reliable propagation, falling well below the established benchmark for success.

The results on Anatomical Similarity Index of the Symbionts (ASIS) proved to be a reliable predictor of long-term graft success. Microscopic observations revealed that successful grafts like 'Anica' and 'Velnița', exhibited rapid restoration of xylem continuity within 30 days. The number and diameter of xylem vessels at the graft interface directly dictate the hydraulic efficiency of the combined biont, and genotypes possessing a higher frequency of large-diameter vessels (60–90 μm) typically demonstrate superior vegetative vigor [70–72]. The horizontal orientation of initial xylem vessels during callus formation is a critical phase in which thermal stress can induce acute grafting shock [43].

A particularly noteworthy anatomical feature observed in this study is the presence of these horizontally oriented xylem vessels at the graft interface. Rather than representing a structural defect, this spatial reorientation serves as a critical, dynamic adaptation during the initial phases of symbiont union. In the first days following chip budding, parenchymatous callus cells proliferate rapidly to bridge the physical gap between the rootstock and scion. As vascular differentiation progresses, newly formed cambial cells must navigate the physical boundaries of the cut surface. The horizontal alignment of these vessels serves as a lateral bridging mechanism, establishing water and nutrient transport before full vertical cambial continuity is achieved.

Our data directly links this anatomical phenomenon to overall grafting success. Experimental variants exhibiting a higher density of these horizontal bridging vessels, most notably chip budding in April (variant a1/b2), demonstrated significantly higher bud burst rates (47.8%) and superior subsequent vegetative vigor. Conversely, variants characterized by delayed or disorganized horizontal vessel formation (such as August patch budding) exhibited restricted hydraulic conductivity, resulting in lower graft take. Using ImageJ, we counted how often these horizontal vessels appeared in April and August samples. Consequently, horizontal vessels were predominantly observed in 68% of the April chip budding variants compared to only 32% in the August patch budding sample. These findings indicate that the efficiency of horizontal vessel differentiation serves as a crucial, early-stage anatomical determinant of graft compatibility and survival in walnut multiplication.

Application of the TOPSIS method [73] integrated diverse parameters, such as biometric growth, ASIS scores, and survival rates, into a single performance metric, thereby identifying the top-performing variants. The consistently high rankings of 'Velnița' and 'Grădinar' across multiple experimental variants suggest that these genotypes possess intrinsic physiological plasticity, making them ideal for intensive nursery production systems. Correspondingly, the CRITIC method highlighted that for summer grafting, rootstock diameter is the primary limiting factor, commanding a weight of 29%. This aligns with the understanding that robust rootstock development is required to support dormant bud survival through the winter season [74].

By contrast, the low performance of the 'Șorogari', 'Săbăuani', 'Bălțăți', and 'Belcești' genotypes underscores a critical challenge in pomology: the complex genotype x environment (G $\times$ E) interaction [75,76]. Unlike more stable genotypes, these four genotypes demonstrated high sensitivity to external inputs, specifically the synergy between technological interventions and the timing of grafting. Reflecting this lack of stability, the TOPSIS rankings suggest that these genotypes possess a narrower optimal window for physiological success. When environmental conditions deviate from this window, whether through delayed grafting or suboptimal nutrient management, the plants fail to maintain homeostatic balance. These genotypes struggled to maintain a physiological balance across all analyzed parameters under varying conditions [77,78], resulting in significant fluctuations in biomass accumulation, sap flow efficiency, and overall yield.

In conclusion, the results of this study demonstrated that while walnut grafting remains technically demanding, the combination of spring chip budding in protected spaces and multi-criteria performance ranking can stabilize production yields to a commercially viable level.

5. Conclusions

The evaluation of walnut grafting performance in a polyethylene high tunnel, using two grafting methods across two distinct execution windows, highlights the high efficiency of the chip budding method. The chip budding method demonstrated clear superiority over patch budding, achieving a significantly higher average success rate. Furthermore, spring grafting (April) proved to be the optimal timing, enhancing both graft establishment and subsequent shoot growth under protected conditions.

Microscopic analysis confirmed complete xylem continuity within 30 days post-grafting, validating the physiological compatibility between the studied local genotypes and the 'Bălțați' rootstock. Additionally, the TOPSIS multi-criteria decision analysis effectively prioritized the experimental variants, identifying April chip budding (variant a1/b2) as the optimal technological solution across all analyzed physiological and morphological parameters.

Among the evaluated cultivars, the genotypes 'Anica', 'Velnița', 'Grădinar', and 'Miroslava' consistently ranked highest. While their exact internal hierarchy varied slightly depending on the specific grafting method and time period, the overall composition of this top tier remained stable. This consistency indicates not only efficient wound healing at the graft union and the successful restoration of vascular continuity, but also a lower sensitivity to external technological and environmental fluctuations.

In conclusion, walnut grafting success depends critically on the synergy between execution timing and plant genetics. Although spring chip budding in April optimizes environmental conditions, the significant variation among the studied genotypes highlights that the scion genotype is equally vital for ensuring high grafting compatibility and long-term propagation efficiency.

Author Contributions: Conceptualization: C.Z., M.F. and M.I.; Methodology: C.Z., M.F., M.D., A.P.S. and M.I.; Investigation: C.Z., R.P. and M.F.; Data curation: C.Z., M.F., M.D. and R.P.; Statistical analysis and validation: M.F., A.P.S. and M.I.; Visualization: C.Z., M.F. and M.I.; Writing—original draft: C.Z., R.P. and M.F.; Writing—review and editing: C.Z., R.P. and M.I.; Supervision and administration: R.P. and C.Z. All authors have read and agreed to the published version of the manuscript.

Funding: The research received no external funding.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the first and corresponding author.

Acknowledgments: This research was funded by "Ion Ionescu de la Brad" Iasi University of Life Sciences. The authors have reviewed and edited the output and take full responsibility for the content of this publication.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

CRITIC	Criteria Importance Through Intercriteria Correlation
TOPSIS	Technique for Order of Preference by Similarity to Ideal Solution
MCDA	Multi-Criteria Decision-Analysis
ASIS	Anatomical Similarity Index of the Symbionts

References

1. Hu, J.; Shi, H.; Zhan, C.; Qiao, P.; He, Y.; Liu, Y. Study on the Identification and Detection of Walnut Quality Based on Terahertz Imaging. *Foods* **2022**, *11*, 3498. [CrossRef]
2. Arab, L.; Guo, R.; Elashoff, D. Lower Depression Scores among Walnut Consumers in Nhanes. *Nutrients* **2019**, *11*, 275. [CrossRef]
3. Achim, G.; Botu, I. Results in walnut propagation by using different methods. *Acta Hortic.* **2001**, *544*, 503–509. [CrossRef]
4. Hartmann, H.T.; Kester, D.E.; Davies, F.T., Jr.; Geneve, R.L. *Plant Propagation. Principles and Practices*; Prentice-Hall: Paramus, NJ, USA, 2002.
5. Porebski, S.; Rzeznicka, B.; Poniedzialek, W. Comparison of two methods of walnut grafting. *J. Fruit Ornam. Plant Res.* **2002**, *10*, 55–62.
6. Karadeniz, T. Relationships between graft success and climatic values in walnut (*Juglans regia* L.). *J. Cent. Eur. Agric.* **2005**, *6*, 631–634.
7. Gandev, S. Budding and grafting of the walnut (*Juglans regia* L.) and their effectiveness in Bulgaria. *Bulg. J. Agric. Sci.* **2007**, *13*, 683–689.
8. Mir, M.; Kumar, A. Effect of different methods, time and environmental conditions on grafting in walnut. *Int. J. Farm. Sci.* **2011**, *1*, 17–22.
9. Pandelea, A.V.; Grădinariu, G.; Zlati, C.; Iurea, E. Studies on biological peculiarities of growth and development graft at different grafting methods. *Sci. Pap. USAMV Iaşi Ser. Hortic.* **2013**, *56*, 207–212.
10. Bernardis, R.; Dascălu, M.; Chelariu, E.L.; Zlati, C.; Pașcu, R.; Posta, D. The analysis of some ornamental rose varieties grown in the green spaces from Iași. *Sci. Pap. USV Ser. B Hortic.* **2022**, *66*, 638–643.
11. Kithi, L.; Lengyel-Kónya, É.; Berki, M.; Bujdosó, G. Role of the green husks of Persian walnut (*Juglans regia* L.) A Review. *Horticulturae* **2023**, *9*, 782. [CrossRef]
12. Ozkan, Y.; Gumus, A. Effects of different applications on grafting under controlled conditions of walnut (*Juglans regia* L.). *Acta Hortic.* **2001**, *544*, 515–520. [CrossRef]

13. Chandel, J.S.; Gautam, D.R.; Sharma, N.C. Chip budding an excellent method of propagation of walnut. *Acta Hortic.* **2006**, *705*, 335–339. [CrossRef]
14. Bai, Y.; Zhang, J.; Wu, Y.; Huang, R.; Chang, Y.; Lei, X.; Song, X.; Pei, D. Possibility of Increasing the Growth and Photosynthetic Properties of Precocious Walnut by Grafting. *Sustainability* **2020**, *12*, 5178. [CrossRef]
15. Ebrahimi, A.; Vahdati, K.; Fallahi, E. Improved success of Persian walnut grafting under environmentally controlled condition. *Int. J. Fruit. Sci.* **2006**, *6*, 3–12. [CrossRef]
16. Nostrati, Z.; Khub, A.K. Effect of different budding methods and tissues on grafting success of walnut. *Hortic. Sci. Technol.* **2014**, *32*, 788–793. [CrossRef]
17. Grădinariu, G.; Zlati, C.; Istrate, M.; Draghia, L. Improvement of grafting union in plum by studying graft anatomy. *Acta Hortic.* **2011**, *923*, 283–290. [CrossRef]
18. Zlati, C.; Grădinariu, G.; Istrate, M.; Draghia, L. Formation of graft union in pear: A histological study by using light microscopy. *J. Am. Pomol. Soc.* **2011**, *65*, 185–191. [CrossRef]
19. Zlati, C.; Grădinariu, G.; Istrate, M.; Draghia, L. Histological investigation on graft formation in pear/quince (*Pyrus communis/Cydonia oblonga*) combinations. *Acta Hortic.* **2011**, *923*, 291–298. [CrossRef]
20. Farsi, M.; Mohammad, R.F.; Zabihollah, Z.; Darab, H.; Ahmad, A. The histology of minigrafting of Persian walnut trees cv. Chandler Summer and Autumn. *Int. J. Hortic. Sci. Technol.* **2016**, *3*, 167–177.
21. Cimpoi, V.I.; Rotaru, L.; Colibaba, L.C.; Scutarasu, E.C.; Calin, I.; Alexandru, C.L. Influence of carbohydrate content on grafting in wine grape varieties 'Aromat De Iași' and 'Golia'. *Sci. Pap. Ser. B Hortic.* **2020**, *64*, 248–254.
22. Fukasawa, R.; Miyazawa, T.; Abe, C.; Bhaswant, M.; Toda, M. Quantification and comparison of nutritional components in Oni walnut (*Juglans ailanthifolia* Carr.), Hime walnut (*Juglans subcordiformis* Dode.), and cultivars. *Horticulturae* **2023**, *9*, 1221. [CrossRef]
23. Vijan, L.E.; Giura, S.; Mazilu, I.C.; Botu, M. Effect of temperature and storage time on some biochemical compounds from the kernel of some walnut cultivars grown in Romania. *Horticulturae* **2023**, *9*, 544. [CrossRef]
24. Mitrović, M.; Miletić, R.; Lukić, M.; Blagojević, M.; Rakićević, M. Impact of rootstock on callus formation in walnut grafted in room conditions. *J. Pomol. Hortic. Sci.* **2008**, *42*, 43–47.
25. Florea, M. Research on the Improvement of the Production Technology of Walnut Grafted Planting Material. Ph.D. Thesis, Ion Ionescu de la Brad Iasi University of Life Sciences, Iasi, Romania, 2022.
26. Dehgan, B.; Vahdati, K.; Rezaee, R.; Hassani, D. Walnut grafting success as affected by different grafting methods, cultivars and forcing treatments. *Acta Hortic.* **2010**, *861*, 345–352. [CrossRef]
27. Hodaj, B.; Rama, P.; Hodaj, N. Time and methods of walnut budding (*Juglans regia* L.). *J. Food Agric. Environ.* **2014**, *12*, 1350–1352.
28. Singh, L.; Awasthi, M.; Negi, P.; Negi, M. Studies on succes rate of grafting methods on walnut (*Juglans regia* L.) at different time under polyhouse condition. *J. Pharmacogn. Phytochem.* **2019**, *8*, 2657–2659.
29. Thapa, R.; Thapa, P.; Ahamad, K.; Vahdati, K. Effect of grafting methods and dates on the graft take rate of Persian walnut in open field condition. *Int. J. Hortic. Sci. Technol.* **2021**, *8*, 133–147.
30. Ni, Z.; Hu, H.; Xu, G. Cold hardiness and physio-biochemical responses of annual branches in five early-fruiting walnut varieties (*Juglans regia* L.) under simulated low-temperature stress. *Horticulturae* **2025**, *11*, 72. [CrossRef]
31. Grădinariu, G.; Istrate, M.; Zlati, C.; Corneanu, G.; Petre, L.; Grădinariu, F.S. The National germoplasm and new sweet cherry and hybrids obtained in Iasi, Romania. *Acta Hortic.* **2007**, *760*, 439–445. [CrossRef]
32. Istrate, M.; Dascălu, M.; Iacob, F.; Zlati, C.; Arcăleanu, A. Agrobiologic potential evaluation of some new sweet cherry varieties in order to improve upon the Romanian assortment. *Sci. Pap. USAMV Iaşi Hortic. Ser.* **2016**, *59*, 201–206.
33. Chelariu, E.L.; Draghia, L.; Brînză, M.; Cojocariu, M.; Avarvarei, B.V. Studies regarding the behavior of ornamental species Lagurus ovatus in cropping conditions from NE area of Romania. *Sci. Pap. Ser. B Hortic.* **2018**, *62*, 581–584.
34. Pașcu, R.; Zlati, C.; Bernardis, R. Rehabilitation of the dendrological park in Buhusi, Bacau County. *Sci. Pap. Ser. B Hortic.* **2022**, *66*, 730–737.
35. Cojocariu, M.; Chelariu, E.L.; Chiruta, C.; Pașcu, R.; Avarvarei, B.V. Comparative study on the behavior of *Plectranthus forsteri* and *Coleus blumei* species growing on the ground and in vertical systems for green façades in the climate of North-East Romania. *Sci. Pap. Ser. B Hortic.* **2022**, *66*, 657–666.
36. Tucaliuc, R.A.; Mangalagiu, V.; Mangalagiu, I.I. Pyridazinic bioisosteres with potential applications in medicinal chemistry and agriculture. *Processes* **2023**, *11*, 2306. [CrossRef]
37. Bernardis, R.; Zlati, C.; Poșta, D. The influence of some growth regulators on the germinability and development at Albizia Julibrissin Durazz. *Sci. Pap. Ser. B Hortic.* **2024**, *68*, 567–574.
38. Ye, Q.; Yue, Q.; Zhang, Y.; Zhang, R.; Jin, Q.; Zhang, J.; Zhu, S.; Zhao, M.; Guo, Z. Impacts of tree thinning on overall productivity in densely planted walnut orchards. *Horticulturae* **2025**, *11*, 1216. [CrossRef]
39. Dascălu, M.; Istrate, M.; Grădinariu, G.; Morariu, A.; Brînză, M.; Ciobotari, G.; Zlati, C.; Căuleţ, R. Variation of structure and xylem conduit dimensions in relation with incompatibility at grafted fruit trees. *Sci. Pap. USAMV Iaşi Hortic. Ser.* **2009**, *52*, 563–568.

40. Zulqarnain, R.M.; Xin, X.L.; Siddique, I.; Asghar Khan, W.; Yousif, M.A. TOPSIS Method Based on Correlation Coefficient under Pythagorean Fuzzy Soft Environment and Its Application towards Green Supply Chain Management. *Sustainability* **2021**, *13*, 1642. [CrossRef]
41. Diakoulaki, D.; Mavrotas, G.; Papayannakis, L. Determining objective weights in multiple criteria problems: The critic method. *Comp. Oper. Res.* **1995**, *22*, 763–770. [CrossRef]
42. Hwang, C.L.; Yoon, K. *Multiple Attribute Decision Making: Methods and Applications*; Springer-Verlag: New York, USA, 1981; pp. 186–259.
43. Santamarina Siurana, M.P.; Roselló Caselles, J.S.; García Breijo, F.J. *Anatomía de las Plantas/The Plant Body: Structure*; Ediciones Mundi-Prensa: Madrid, Spain, 2018.
44. Duncan, D.B. Multiple Range and Multiple F Tests. *Biometrics* **1955**, *11*, 1–42. [CrossRef]
45. Săulescu, N.A.; Săulescu, N.N. *Câmpul de Experienţă/Experimental Field*, 2nd ed.; Editura Agro-Silvică: Bucureşti, Romania, 1967.
46. Mladin, G.; Petrescu, S.; Butac, M. Rezultate preliminare privind unele elemente morfo-fiziologice implicate în convieţuirea simbionţilor soi-portaltoi la cireş/Preliminary results regarding some morpho-physiological elements involved in the coexistence of cultivar-rootstock symbionts in cherry. *Fruit. Grow. Res.* **2006**, *22*, 182–189.
47. Arghavan, S.; Ganji-Moghadam, E.; Fahadan, A.; Zamanipour, M. Possibility of early detection of graft incompatibility in some commercial plum cultivars by phenolic compounds analysis. *J. Hort. Sci.* **2022**, *17*, 488–495.
48. Chiruţă, C.; Stoleriu, I.; Cojocariu, M. Prediction models for the plant coverage percentage of a vertical green gall system: Regression models and artificial neural network models. *Horticulturae* **2023**, *9*, 419. [CrossRef]
49. Cojocariu, M.; Marta, A.E.; Jităreanu, C.D.; Chelariu, E.L.; Căpşună, S.; Cara, I.G.; Amișculesei, P.; Istrate, A.M.R.; Chiruţă, C. A study on the development of two ornamental varieties of Ipomoea batatas cultivated in vertical systems in the Northeastern region of Europe. *Horticulturae* **2024**, *10*, 133. [CrossRef]
50. Seyedmohammadi, J.; Sarmadian, F.; Jafarzadeh, A.A.; Ghorbani, M.A.; Shahbazi, F. Application of SAW, TOPSIS and Fuzzy TOPSIS models in cultivation priority planning for maize, rapeseed and soybean crops. *Geoderma* **2018**, *310*, 178–190. [CrossRef]
51. Ćirković-Mitrović, T.; Brašanac-Bosanac, L.; Popović, V.; Ivetić, V. Morphological indicators of quality of Turkish hazel (*Corylus colurna* L.) one-year-old seedlings. In Proceedings of the Sixth International Scientific Agricultural Symposium "Agrosym 2015", Jahorina, Bosnia and Herzegovina, 15–18 October 2015; pp. 2100–2106.
52. Mohamed, A.; Stokes, A.; Mao, Z.; Jourdan, C.; Sabatier, S.; Pailler, F.; Fourtier, S.; Dufour, L.; Monnier, Y. Linking above-and belowground phenology of hybrid walnut growing along a climatic gradient in temperate agroforestry systems. *Plant Soil* **2018**, *424*, 103–122. [CrossRef]
53. Rezaee, R.; Vahdati, K.; Grigoorian, V.; Valizadeh, M. Walnut grafting success and bleeding rate as affected by different grafting methods and seedling vigour. *J. Hortic. Sci. Biotechnol.* **2008**, *83*, 94–99. [CrossRef]
54. Paunović, S.M.; Milinković, M.; Karaklajić-Stajić, Ž.; Tomić, J.; Rilak, B. Production of walnut grafts under controlled conditions and plants development in the nursery. In Proceedings of the 6th International Symposium "Modern Trends in Agricultural Production, Rural Development and Environmental Protection", Vrnjačka Banja, Serbia, 27–28 June 2024; pp. 129–137.
55. Zulqarnain, R.M.; Xin, X.L.; Saeed, M. Extension of TOPSIS method under intuitionistic fuzzy hypersoft environment based on correlation coefficient and aggregation operators to solve decision making problem. *Aims Math.* **2020**, *6*, 2732–2755. [CrossRef]
56. Han, Q.; Li, W.; Song, Y.; Zhang, T.; Wang, R. A new method for MAGDM based on improved TOPSIS and a novel pythagorean fuzzy soft entropy. *Symmetry* **2019**, *11*, 905. [CrossRef]
57. Zenginbal, H. The Effects of different grafting methods on success grafting in different Kiwifruit (*Actinidia deliciosa*, A. Chev) cultivars. *Int. J. Agric. Res.* **2007**, *2*, 736–740. [CrossRef]
58. Rasool, A.; Mansoor, S.; Bhat, K.M.; Hassan, G.I.; Baba, T.R.; Alyemeni, M.N.; Alsahli, A.A.; El-Serehy, H.A.; Paray, B.A.; Ahmad, P. Mechanisms underlying graft union formation and rootstock scion interaction in horticultural plants. *Front. Plant Sci.* **2020**, *11*, 590847. [CrossRef]
59. Sedaghathoor, S.; Noie, M. Study on different grafting methods of Kiwifruit 'Hayward' on the 'Matua' and 'Bruno' rootstocks. *Fruits* **2016**, *71*, 275–280. [CrossRef]
60. Atkinson, C.J.; Else, M.A.; Taylor, L.; Dover, C.J. Root and stem hydraulic conductivity as determinants of growth potential in grafted trees of apple (*Malus pumila* Mill.). *J. Exp. Bot.* **2003**, *54*, 1221–1229. [CrossRef] [PubMed]
61. Tedesco, S.; Erban, A.; Gupta, S.; Kopka, J.; Fevereiro, P.; Kragler, F.; Pina, A. The Impact of Metabolic Scion-Rootstock Interactions in Different Grapevine Tissues and Phloem Exudates. *Metabolites* **2021**, *11*, 349. [CrossRef]
62. Villa-Llop, A.; Buesa, I.; Velaz, M.; Loidi, M.; Escalona, J.M.; Sabater, A.; Santesteban, L.G.; Torres, N. Exploring the Impact of Vascular Alignment and Grafting on Grapevine Physiology and Growth. *Physiol. Plant.* **2026**, *178*, e70704. [CrossRef] [PubMed]
63. Assunção, M.; Santos, C.; Brazão, J.; Eiras-Dias, J.E.; Fevereiro, P. Understanding the Molecular Mechanisms Underlying Graft Success in Grapevine. *BMC Plant Biol.* **2019**, *1*, 396. [CrossRef]
64. Butac, M.; Zamfirescu, B.; Nicolae, S. Results regarding compatibility of some plum cultivars grafted on different rootstocks. *Rom. J. Horic.* **2023**, *4*, 77–84. [CrossRef]

65. Bijelić, S.; Magazin, N.; Džankić, S.; Janković, D.; Bogdanović, B.; Jaćimović, G. Advances in nursery production of hazelnut plants in Serbia-Successful grafting of different *Corylus avellana* L. cultivars and clones onto *Corylus colurna* L. rootstock. *Front. Plant Sci.* **2021**, *12*, 785015. [CrossRef]
66. Milošević, T.; Milosevic, N.; Moreno, M.A.; Mladenović, J. Tree performances of eight rootstocks grafted with 'Šumadinka' sour cherry. *Span. J. Agric. Res.* **2024**, *22*, 6. [CrossRef]
67. Wani, R.A.; Baba, J.A.; Zaffar, G.; Hakeem, S.A.; Umar, I.; Mir, M.A.; Zubair, M. Grafting-take success in walnut (*Juglans regia*) under different environment conditions. *Int. J. Curr. Microbiol. App. Sci.* **2017**, *6*, 2195–2201. [CrossRef]
68. Kumar, N.; Sharma, A.; Lanzes, T.; Jasrotia, A.; Bhat, D.J.; Mir, M.; Das, S. Optimizing propagation techniques and timing for pecan (*Carya illinoinensis*) under Poonch condition of Jammu region. *Indian. J. Agric. Sci.* **2025**, *95*, 938–942. [CrossRef]
69. Khan, S.Q.; Iqbal, U.; Ur Rehman, M.; Mir, M.M.; Mir, M.A.; Parveze, M.U.; Shah, H.K. Anatomical and Histological Investigation of Antioxidants and IBA on Graft Success in Walnut. *Appl. Fruit. Sci.* **2025**, *67*, 54. [CrossRef]
70. Tyree, M.T.; Ewers, F.W. The hydraulic architecture of trees and other woody plants. *New Phytol.* **1991**, *119*, 345–360. [CrossRef]
71. Avanzato, D.; Atefi, J. Environmental and physiological factors affecting callus formation in walnut. *Acta Hortic.* **1997**, *442*, 291–294. [CrossRef]
72. Sadeghi Majd, R.; Vahdati, K.; Roozban, M.R.; Arab, M. Exploring combinations of graft cover and grafting method in commercial walnut cultivars. *Int. J. Fruit. Sci.* **2019**, *19*, 359–371. [CrossRef]
73. Vahdati, K.; Sadeghi-Majd, R.; Sestras, A.F.; Licea-Moreno, R.J.; Peixe, A.; Sestras, R.E. Clonal Propagation of Walnuts (*Juglans* spp.): A Review on Evolution from Traditional Techniques to Application of Biotechnology. *Plants* **2022**, *11*, 3040. [CrossRef]
74. Paunovic, S.M.; Miletic, R.; Mitrovic, M.; Jankovic, D. Effect of callusing conditions on grafting success in walnut (*Juglans regia* L.). *J. Fruit. Ornam. Plant Res.* **2011**, *19*, 5–14.
75. Warschefsky, E.J.; Klein, L.L.; Frank, M.H.; Chitwood, D.H.; Miller, A.J. Rootstocks: Diversity, domestication, and impacts on shoot phenotypes. *Trends Plant Sci.* **2016**, *21*, 418–437. [CrossRef]
76. Albacete, A.; Martínez-Andújar, C.; Martínez-Pérez, A.; Thompson, A.J.; Dodd, I.C.; Pérez-Alfocea, F. Unravelling rootstock× scion interactions to improve food security. *J. Exp. Bot.* **2015**, *66*, 2211–2226. [CrossRef]
77. Chaves, M.M.; Zarrouk, O.; Francisco, R.; Costa, J.M.; Santos, T.; Regalado, A.P.; Rodrigues, M.L.; Lopes, C.M. Grapevine under deficit irrigation: Hints from physiological and molecular data. *Ann. Bot.* **2010**, *105*, 661–676. [CrossRef]
78. Lu, X.; Liu, W.; Wang, T.; Zhang, J.; Li, X.; Zhang, W. Systemic Long-Distance Signaling and Communication Between Rootstock and Scion in Grafted Vegetables. *Front. Plant Sci.* **2020**, *11*, 460. [CrossRef] [PubMed] [PubMed Central]