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ORIGINAL ARTICLE

Unveiling Key Factors in Walnut Grafting Success: The Impact of Grafter Skill and Budding Method

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K E Y W O R D S

Flute budding;
Grafter expertise;
Grafting success;
Persian walnut;
Vegetative propagation

A B S T R A C T

Achieving reliable grafting success in Persian walnut (*Juglans regia* L.) remains challenging due to complex environmental and technical factors. This study examines how the skill of the grafter and the budding technique affect grafting results, offering valuable insights for enhancing walnut propagation practices. We compared two budding methods—patch and flute—applied by grafters with varied experience levels. Results reveal that flute budding, performed by experienced grafters, significantly enhanced callus formation quality, bud take, and overall graft survival rates. The flute method yielded a 94.4% bud-take rate and a 90% graft survival, outperforming patch budding, which achieved 90% and 81.1%, respectively. Notably, the combination of flute budding and the most skilled grafter reached a 100% survival rate, underscoring the impact of technique and expertise on propagation success. These findings highlight grafter proficiency as a key factor in walnut grafting, suggesting that targeted training and the adoption of flute budding could improve propagation efficiency. This study contributes valuable knowledge to walnut propagation, proposing straightforward protocols to enhance the success of vegetative propagation in commercial and research settings.

Introduction

The Persian walnut (*Juglans regia* L.) is a highly prized tree cultivated worldwide due to its exceptional nutritional profile (Habibie *et al*, 2021; Rébufa *et al*., 2022; Habibi *et al*, 2024). Until recent decades, sexual propagation by seed was the primary method farmers relied on for walnut propagation. However, this approach posed significant challenges for walnut production, including a lengthy juvenile phase of 7 to 10 years, low productivity, and uncontrollable tree sizes (Chalise *et al*., 2021). Ensuring consistent, high-quality walnut production relies heavily on vegetative propagation techniques. However, walnuts are more challenging to propagate through tissue culture and grafting compared to many other fruit trees (Vahdati, 2003; Vahdati *et al*., 2008; Rezaee *et al*., 2008; Liu *et al*., 2018). The success of walnut grafting is influenced by a complex set of factors, including environmental conditions such as temperature and relative humidity, choice of grafting method, coverage of the grafting site,

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timing, and the vigor and compatibility of rootstock and scion (Sadeghi-Majd *et al*., 2019, 2022).

Research indicates that the optimal temperature for callus formation at the graft site is between 26–27°C, with a recommended relative humidity above 80% to achieve successful walnut grafting (Vahdati *et al*., 2022; Mostakhdemi *et al*., 2022). Consequently, selecting the appropriate timing for walnut grafting, specific to each region, is essential for maximizing success. The grafting method itself plays a significant role in graft success, and numerous global studies have evaluated various techniques, revealing substantial differences in outcomes based on the method used (Atefi, 1997; Vahdati and Zarei, 2006; Ozkan *et al*., 2006; Dehghan *et al*., 2010; Nosrati *et al*., 2014). To promote optimal moisture levels and grafting success, covering the grafting site with moisture-retentive materials has become a standard practice (Rezaee *et al*., 2008; Sadeghi-Majd *et al*., 2019). However, the challenge of grafting in walnuts gradually evolved with the introduction of more advanced techniques, such as hot callus, micro-grafting, and epicotyl grafting, which brought some success (Ribeiro *et al*, 2022; Liu *et al*, 2023).

While some studies suggest that genotype has little influence on grafting success, others report it to be a significant factor (Ozkan *et al*., 2001; Khalil *et al*., 2009; Dehghan *et al*., 2010; Ertürk, 2013; Sadeghi-Majd *et al*., 2019). Anyway, grafting is a widely used propagation method in fruit tree production and breeding. It has been employed in numerous studies to enhance the yield, quality, and resistance of horticultural crops, as well as to investigate the long-distance transport of molecules. However, the underlying mechanisms of graft union formation (GUF) remain poorly understood (Song *et al*, 2024, Lou *et al*, 2024). In an in situ study, the spatial and temporal distribution of endogenous hormones (IAA, ABA, and ZR) during walnut GUF was examined. The findings revealed an increase in endogenous IAA and ZR levels during the rapid proliferation phase of callus tissue, with enrichment observed in the cambium and cells at the grafting interface. As the callus tissue transitioned into the differentiation stage, endogenous IAA and ZR levels decreased rapidly and were predominantly localized in the callus tissue. Conversely, the levels and distribution of endogenous ABA showed little change during the rapid proliferation phase but increased significantly during the differentiation stage, mainly in the scion cambium and callus tissue (Song *et al*, 2024). The impact of grafter skill—a potentially crucial yet understudied factor—on grafting success has received limited attention. This study addresses this gap by evaluating the influence of grafter expertise, with three professionals of varying experience levels, on walnut grafting outcomes using two budding techniques: patch and flute budding.

Material and Methods

Experimental site and plant materials

The experiment was conducted from April 2021 to October 2021 at the College of Aburaihan of the University of Tehran in Pakdasht, Tehran, Iran, under field conditions in the nursery. The soil texture at the site was silty loam with a pH of 7.3. Half-sib seeds of early-bearing walnut genotypes, planted in 2019, were utilized in a randomized block design with three replications. Each replication consisted of 10 grafts with the 'Chandler' cultivar, budded on April 1, 2021, utilizing two grafting methods (flute and patch budding) performed by three grafters (Fig. 1A). One grafter possessed three years of experience, while the other two had over ten years of skills and experience. Prior to budding, branches of the 'Chandler' cultivar were collected, wrapped in a damp cloth, covered with plastic wrap, and stored in a refrigerator at 4°C until the budding process. Budding was carried out at a height of 15 cm from the soil surface.

Fig. 1. A: Walnut nursery at the Department of Horticulture, College of Aburaihan, University of Tehran, Pakdasht, Tehran, Iran. Callus formation at the budding site of flute budding (B) and patch budding (C).

Budding methods

1. Patch grafting: a plant budding technique in which a small piece of bark containing a scion bud is inserted into a corresponding opening in the rootstock.

2. Flute grafting: a budding method involving the complete removal of a piece of bark (with a 3.5 cm ring), with the bud covering the rootstock wound.

Post-budding procedures

One week following budding, the last third of the rootstock was removed. Following three weeks from the budding date, coinciding with sufficient callus formation at the grafting site, the rootstock seedling was cut about half a centimeter above the budding site, allowing only the scion to continue growing.

Data collection

The following parameters were assessed for data collection:

- Callus formation/quality: characterized by the percentage of callus formation at the grafting point (ratings: 1 - very low, 2 - low, 3 - medium, 4 - good, 5 - very good) three weeks post-budding.

- Bud-take (%): the percentage of seedlings with green and fresh scions three weeks after budding.

- Bud survival (%): the percentage of trees with fused bud sites by the end of the growing season, indicating healthy graft growth.

- Scion diameter (mm): measured with calipers twenty centimeters above the grafting point at the conclusion of the growing season.

- Scion height (cm): the height of shoot growth from the budding point to the highest point of scion growth at the end of the growing season.

Data analysis

The data were analyzed using SAS software (version 1.6). Means were compared using Duncan's multiple range tests (DMRT) at a significance level of $P \leq 0.05$.

Results

The data analysis revealed significant impacts of the budding method and grafter skills on budding success. Mean comparisons indicated that the flute grafting method exhibited superior callus quality conditions with a mean score of 3.66 compared to 3.38 for patch budding (Figs. 1B, 1C, 2A). While there was a slight disparity in bud-take between the two methods, the flute technique demonstrated a notably higher success rate at 94.44% in contrast to 90% for patch budding (Fig. 2B). Additionally, flute budding showcased a higher survival rate of 90% compared to 81.11% for patch budding. Furthermore, in terms of morphological traits such as scion growth and scion diameter, flute grafting resulted in the production of trees with superior quality for marketability (Figs. 2C to 2E).

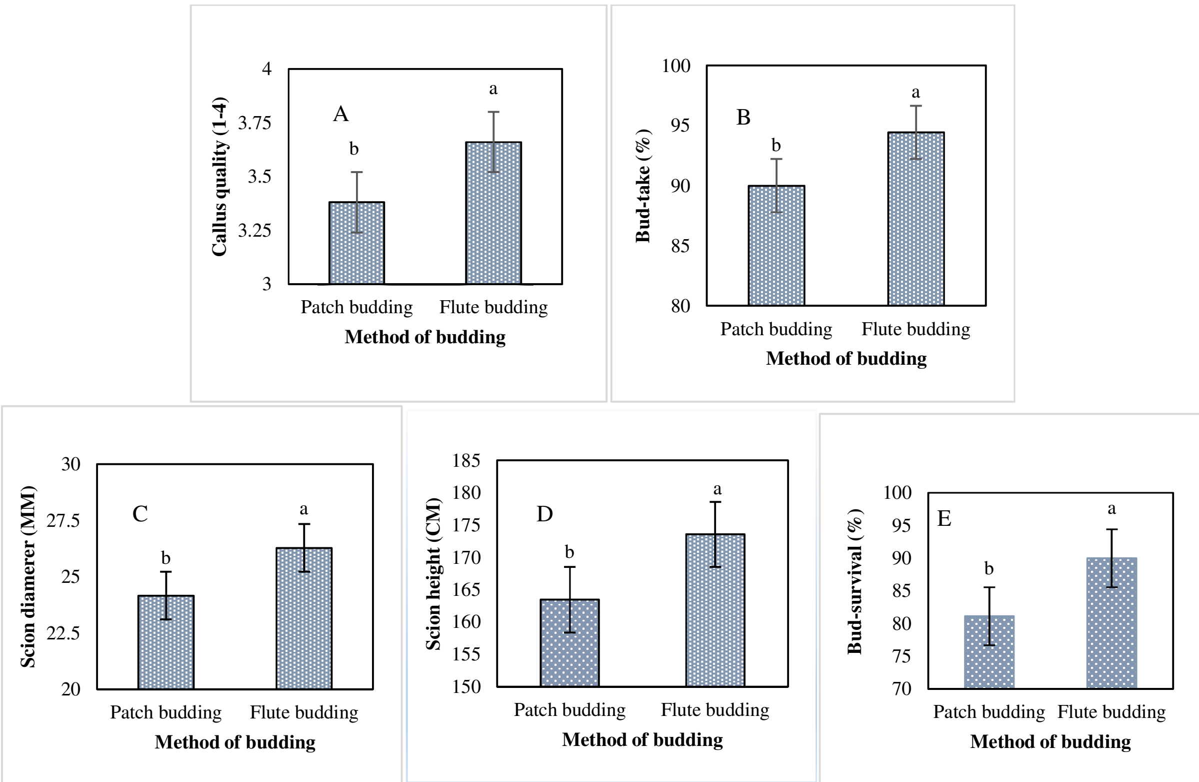

Fig. 2. The impact of flute and patch budding methods on callus quality (A), bud-take (B), scion diameter (C), scion growth (D), and bud survival (E). Bud survival calculated as (S/B) x 100, where S represents the number of surviving plants, and B denotes the number of bud-takes. Values with different letters denote statistical significance.

The influence of grafter skills on the studied traits exhibited significant differences in callus quality, bud-take, scion diameter/growth, and bud survival rates (Figs. 3A to 3E). Notably, the skills and experience level of grafter 1 yielded the lowest values across all assessed parameters, emphasizing the positive correlation between grafter experience and budding success (Fig. 3A to 3E).

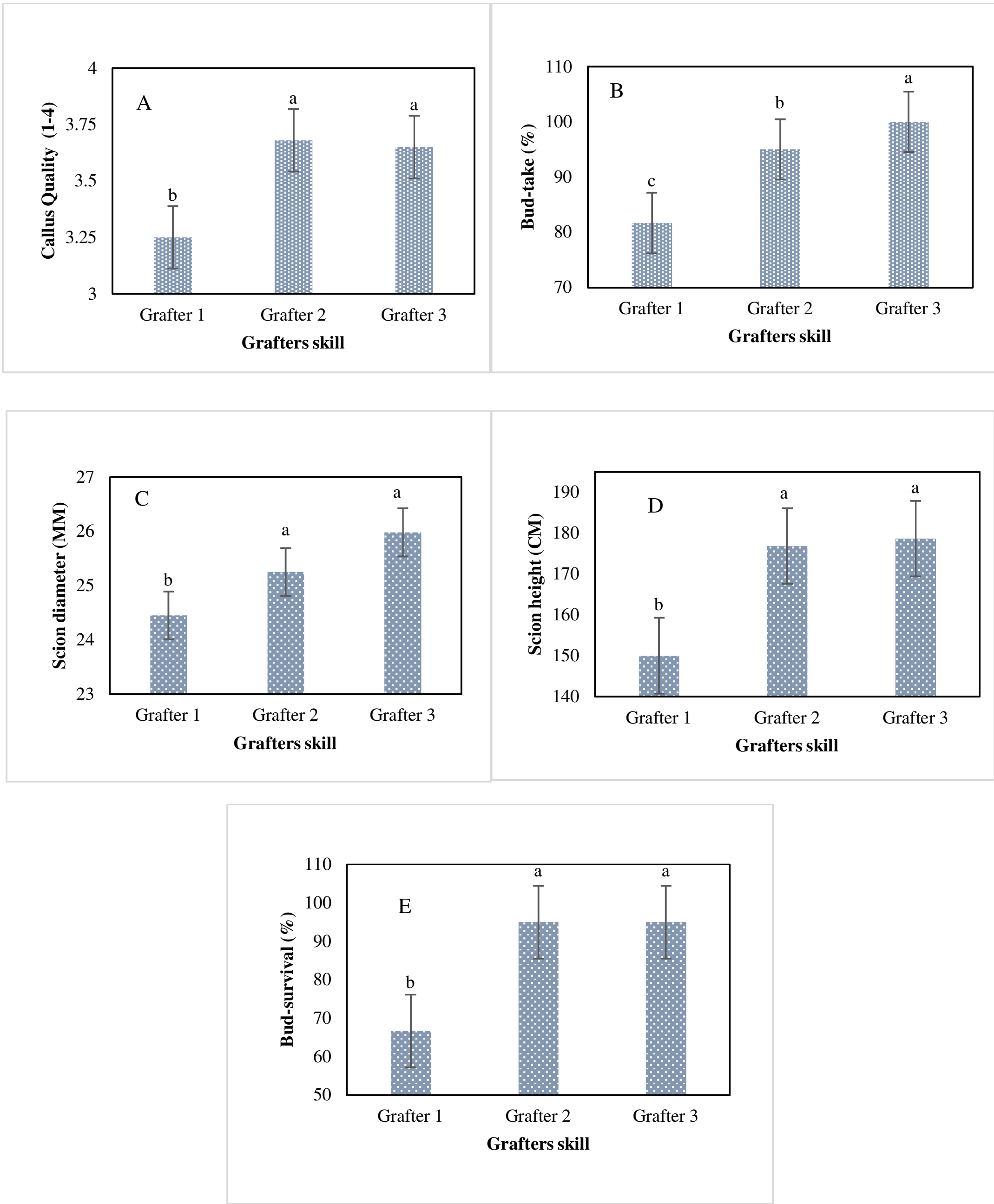

Fig. 3. The influence of grafter's skill on callus quality (A), bud-take (B), scion diameter (C), scion growth (D) and bud survival (E). Bud survival calculated as (S/B) x 100, where S represents the number of surviving plants, and B denotes the number of bud-takes. Values with different letters denote statistical significance.

Interactions between Grafters' skills and budding methods

The interaction analysis between grafter skills and budding methods revealed varying outcomes. The highest and lowest callus quality scores of 3.83 and 3.06 out of 4 were attributed to grafter 2 with flute buds and grafter 1 with patch buds, respectively (Fig. 4). In terms of bud-take, grafter 1 displayed the lowest rates across all combinations, while grafter 2 achieved the highest success rates both with flute and patch budding methods (Fig. 5). Grafter 2's fluted budding was associated with 100% success. The interaction between grafter skills and budding methods also significantly impacted scion height and diameter. Grafters' skills and grafting methods influenced scion growth, with grafter 1 exhibiting the smallest shoot diameter and scion height in patch budding, while flute budding resulted in larger scion dimensions (Figs. 6 & 7). Survival rates, a crucial commercial trait, were strongly influenced by the budding method and grafter skills, with grafter 1 recording the lowest success rates across all traits studied. Conversely, grafters 2 and 3 achieved notable successes, with 100% survival in flute budding and 90% in patch budding (Fig. 8).

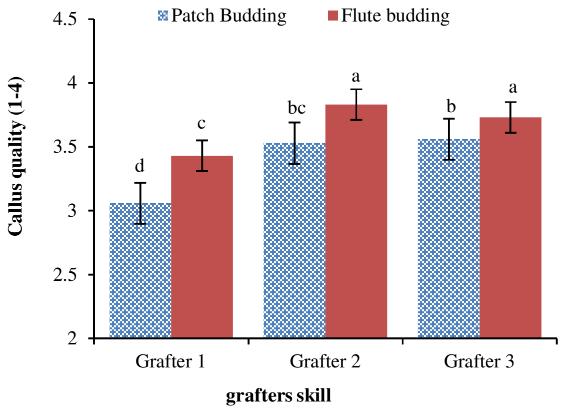

Fig. 4. Interactions between grafter's skill and budding methods on callus quality. The values represent the mean of the callus scoring ranging from 1 (low callus formation) to 4 (very good callus formation).

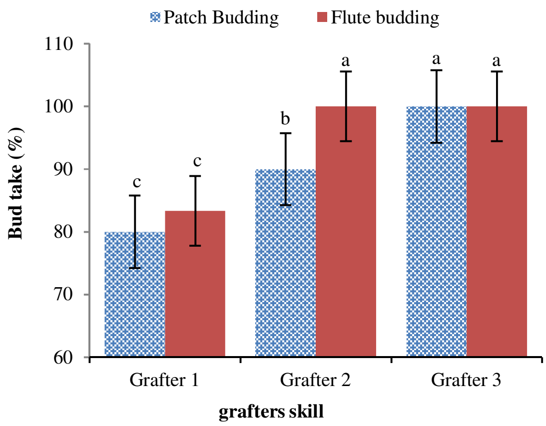

Fig. 5. Interactions between grafter's skill and budding methods on bud-take (%).

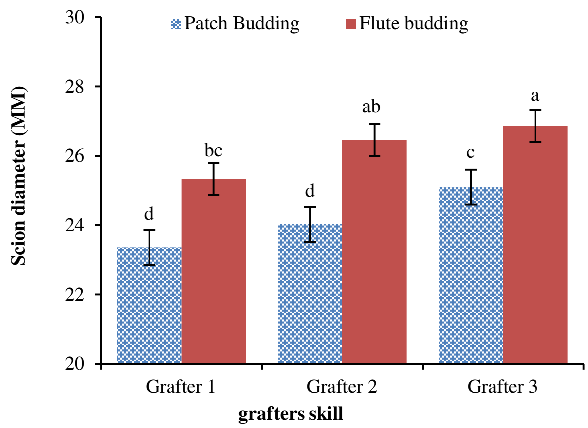

Fig. 6. Interactions between grafter's skill and budding methods on scion diameter (mm).

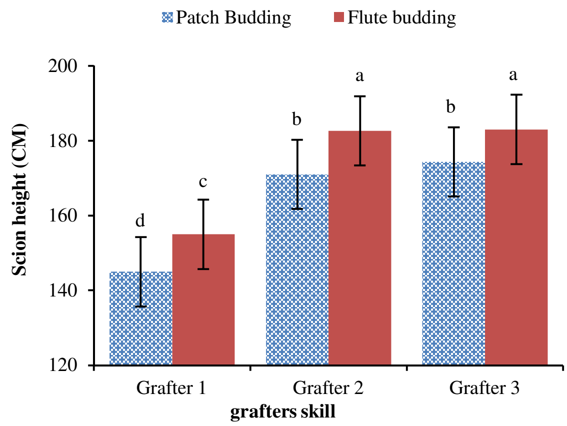

Fig. 7. Interactions between grafter's skill and budding methods on scion height (cm).

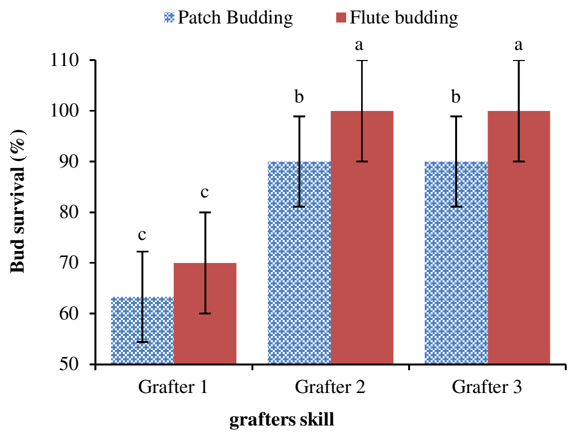

Fig. 8. Interactions between grafter's skill and budding methods on graft survival.

Discussion

Our study aimed to assess the impact of budding methods and grafter skills on various parameters such as callus formation, bud take, scion diameter and growth, and scion survival rate. The results demonstrated a significant difference in outcomes based on the budding method employed and the skills of the grafter. Notably, flute budding outperformed patch budding, with survival rates reaching 90% for flute budding compared to 81.11% for patch budding (Fig. 2E). These findings align with previous research by Ghamari Hesabi *et al*. (2016), Nosrati *et al*. (2014), Singh *et al*. (2019), and Thapa *et al*. (2021), highlighting the critical influence of grafting methods on overall success rates. Ebrahimi *et al* (2007) and Vahdati *et al* (2022) demonstrated superior results with patch budding compared to others budding methods.

Ebrahimi *et al*. (2007) achieved the highest grafting success with patch budding, reporting a success rate of 91%, compared to 31.1% for shield budding and 19.1% for chip budding.

Ghamari Hesabi *et al*. (2016) noted that for successful budding, the rootstock must be strong and well-watered to ensure the bark can be easily separated from the wood. Therefore, by controlling environmental conditions and performing the grafting or budding at the optimal time, grafting success can be significantly improved (Vahdati *et al*., 2022).

Mean comparisons (P ≤ .05) underscored the substantial impact of grafter skills on all assessed traits (Fig. 3A-E).

Despite the presence of suitable rootstocks, scions, and optimal grafting conditions, the proficiency of the grafter emerged as the most crucial factor influencing grafting success. The interactions observed between budding method and grafter skills further emphasized the joint influence of these factors on the outcomes (Fig. 4-8).

Grafters with greater experience, such as grafter 2 and 3, were able to achieve higher quality callus formation at the budding point (Fig. 4). This variation in callus quality is attributed to the differing degrees of overlap between the cambial layers of the rootstock and scion with varied grafting techniques, as noted in studies by Farsi *et al*. (2018), Sadeghi Majd *et al*. (2021), and Vahdati *et al*. (2022), corroborating our findings.

Our results also highlighted the impact of budding methods and grafter skills on bud take scion diameter, growth, and survival rate (Figs. 4-8). The expertise of the grafter emerged as a pivotal factor in determining the success of grafting procedures, underscoring the importance of practice and experience in enhancing success rates. While grafter skills are essential, it is crucial to acknowledge the role of other variables such as environmental conditions and rootstock-scion interactions in influencing grafting success (Hartmann *et al*., 2002; Dehghan *et al*., 2010; Gandev and Arnaudov, 2011; Ghamari Hesabi *et al*., 2016).

An experiment was conducted to evaluate the effect of grafter skill on the success of whip grafting in mango (Mng'omba *et al*, 2010).

Grafter skill was categorized into three levels: more than ten years of experience, up to three years of experience, and less than one month of experience (unskilled). The results revealed a highly significant impact of grafter skill on grafting success. It was concluded that the ability of grafters to accurately align and securely fasten the cambium tissues of the rootstock and scion was one of the most critical factors for achieving high grafting success (Mng'omba *et al*, 2010).

Although patch budding is often regarded as a successful method for walnut propagation and has been extensively studied in previous research (Ebrahimi *et al*., 2007; Vahdati *et al*., 2022), our findings shed light on the potential success of flute budding for walnuts. While flute budding is traditionally used for pistachios and has been less popular for walnuts due to perceived inefficacy, our study revealed promising results, with 100% survival success achievable under optimal conditions. By performing flute budding at the right time on suitable rootstocks and scions, growers can potentially enhance grafting success rates in walnut propagation.

While grafting methods and optimal timings for walnut have been well-established and have improved fruit production (Dehghan *et al*., 2010, Vahdati *et al*., 2022; Sadeghi-Majd *et al*, 2022), the widespread replacement of diverse genotypes through top-working with a limited number of known cultivars has significantly reduced walnut germplasm diversity. Market pressures have further accelerated the transition from naturally seed-propagated walnut trees to grafted cultivars, intensifying the challenges of conserving walnut genetic resources due to the erosion of genetic diversity caused by grafting (Liu *et al*., 2023).

Consequently, preserving walnut germplasm in dedicated collections has become essential.

Conclusions

This study aimed to establish straightforward, cost-effective protocols for walnut grafting under various conditions, focusing on an often overlooked but crucial factor—the skill level of the grafter. Our findings highlight the significant impact of grafter proficiency on grafting success, with a notable 30% increase in survival rates corresponding to higher levels of expertise. Additionally, we compared two budding methods: the traditional patch budding and the less conventional flute budding. The results revealed that flute budding, when performed by experienced grafters, achieved an exceptional 100% survival rate, demonstrating its considerable potential for improving walnut propagation. However, a challenge with this method was its slower operational pace compared to patch budding, which may influence its practical application.

These results underscore the critical role of grafter skill in determining grafting success. Furthermore, the positive outcomes associated with flute budding present promising prospects for enhancing the efficiency of walnut propagation. We recommend that future research focus on further refining grafting techniques, optimizing budding methods, and exploring the dynamic interaction between grafter skill and method selection to continue advancing the field of walnut horticulture.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

Credit author statement

Rasoul Sadeghi-Majd: Conceptualization, methodology, investigation, writing – original draft; Mahmoud Reza Roozban: Conceptualization, methodology, supervision, review & editing; Saadat Sarikhani: conceptualization, review & editing; Maryam Norouzi: Supervision, review & editing. Kourosh Vahdati: Conceptualization, methodology, review & editing, funding acquisition, project administration.

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Conflict of interests

The authors declare no competing interests.

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