IECAG 2025 Conference

The 5th International Electronic Conference on Agronomy

15–18 December 2025 | Online

MDPI

New insights on the production of grafted walnut plants

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INTRODUCTION & AIM

- Commercial walnut production still depends on field or greenhouse grafting using seedling rootstocks, a slow approach that contributes to orchard variability and limits mechanization.
- *In vitro* micropropagation has enabled clonal multiplication of walnut cultivars, but grafting practices remain largely traditional. *In vitro* micrografting offers a faster and more uniform alternative by combining *in vitro*–produced rootstocks and scions.
- This study aimed to evaluate the potential of *in vitro* micrografting as a replacement for conventional methods, and compare two cleft grafting types using 'Paradox' cl. 'Vlach' rootstocks with 'Chandler' and 'Howard' scions, and determine how different rootstock developmental stages influence grafting success to identify the most effective method and cultivar–stage combination.

METHOD

Plantlets of the hybrid rootstock Paradox cl. 'Vlach' (*Juglans regia* × *Juglans hindsii*) and the cultivars 'Chandler' and 'Howard' (*J. regia*) were cultured *in vitro* and propagated on DKW culture medium (Driver & Kuniyuki, 1984), following the procedure previously described by Ribeiro et al. (2022) (Figure 1).

Two types of split grafting—top cleft and side cleft—were performed using rootstocks at three developmental stages: pre-rooting, post-rooting, and post-acclimatization. Following the grafting procedure, all plantlets were maintained under identical controlled conditions for 21 days (Figure 2).

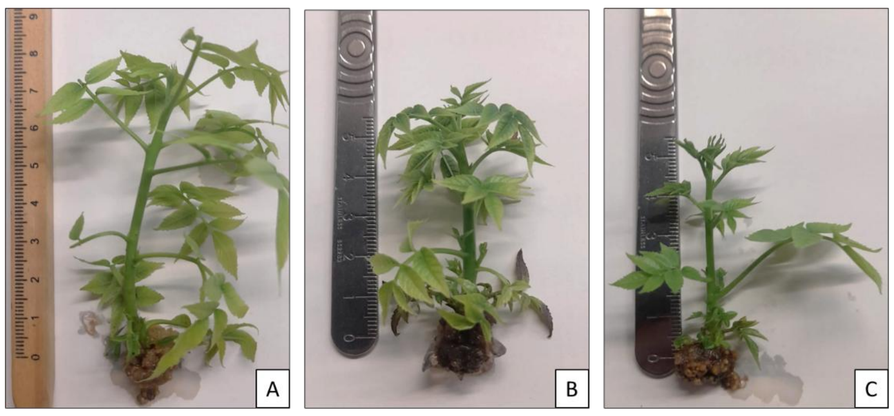

Figure 1. Hybrid 'Paradox' (*Juglans regia* x *Juglans hindsii*) clone 'Vlach' (A), cv. 'Howard' (*J. regia*) (B), cv. 'Chandler' (*J. regia*) (C).

WORKFLOW

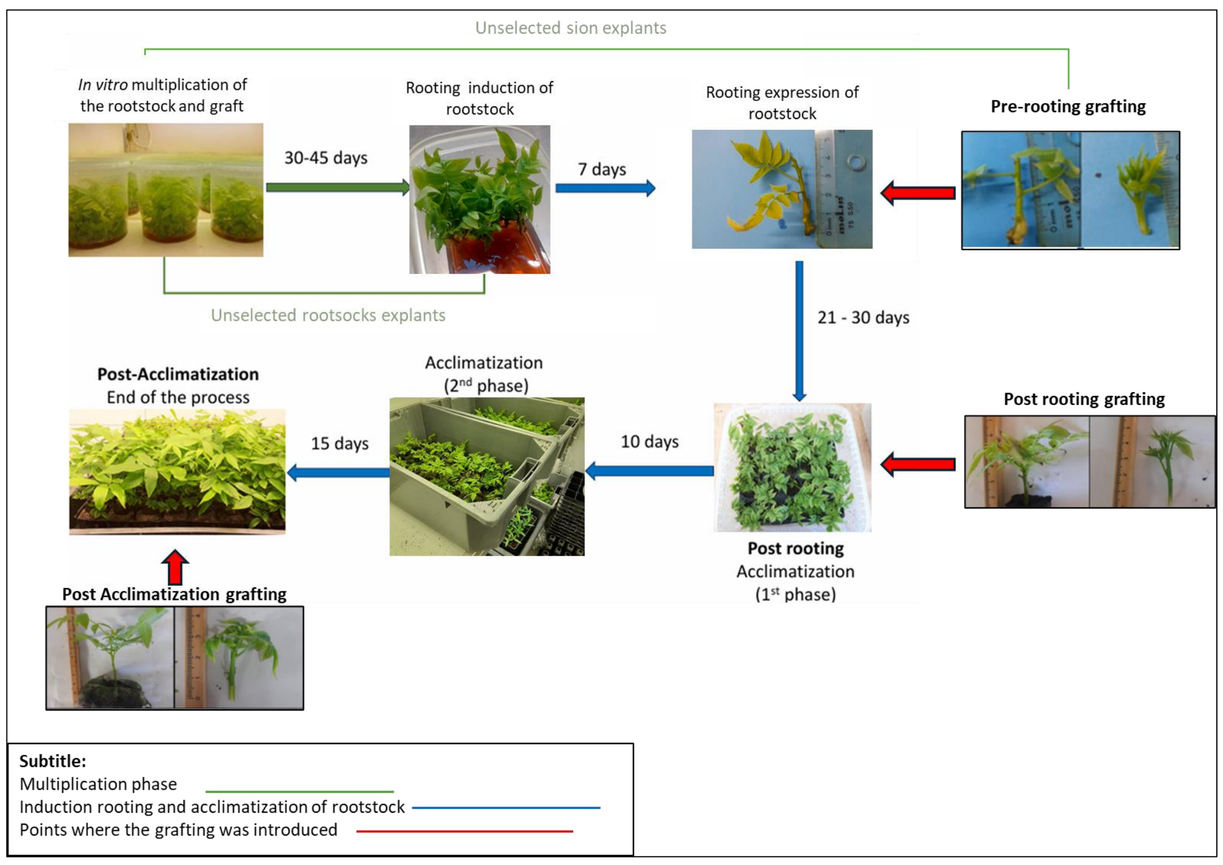

Figure 2. Representative scheme of the essay carried out. Using two walnut cultivars, two micrografting methods (top and lateral) were performed on non-rooted (pre-rooting), rooted but not acclimatized (post-rooting), and already acclimatized rootstocks (post acclimatization).

RESULTS & DISCUSSION

The grafting technique and the scion cultivar did not significantly affect callus formation or grafting success.

Table 1 - Comparison of callus formation rate and grafting success rate between different phases for each cultivar x grafting methods. Values represent the mean (%) ± standard error. Statistically significant differences are considered for $p \leq 0.05$.

Cultivar	Grafting	Callus Formation (%)			Grafting Sucess (%)		
		Pre-rooting	Post-rooting	Post-acclimatization	Pre-rooting	Post-rooting	Post-acclimatization
'Howard'	Top	33,3 ± 8,8^{a}	30,0 ± 5,8^{a}	0,0 ± 0,0^{a}	30,0 ± 5,8^{a}	23,3 ± 3,3^{ab}	0,0 ± 0,0^{b}
	Lateral	56,7 ± 17,6^{ab}	86,7 ± 3,3^{a}	0,0 ± 0,0^{b}	63,3 ± 17,6^{a}	26,7 ± 8,8^{ab}	0,0 ± 0,0^{b}
'Chandler'	Top	46,7 ± 6,7^{a}	10,0 ± 5,8^{b}	23,3 ± 3,3^{ab}	53,3 ± 8,8^{a}	10,0 ± 5,8^{b}	23,3 ± 3,3^{ab}
	Lateral	46,7 ± 12,0^{a}	23,3 ± 8,8^{a}	3,3 ± 3,3^{a}	50,0 ± 5,8^{a}	20,0 ± 10,0^{ab}	3,3 ± 3,3^{b}

Callus formation showed no significant differences among rootstock phases, whereas grafting success was significantly higher in the pre-rooting *in vitro* phase than in the post-rooting and post-acclimatization phases.

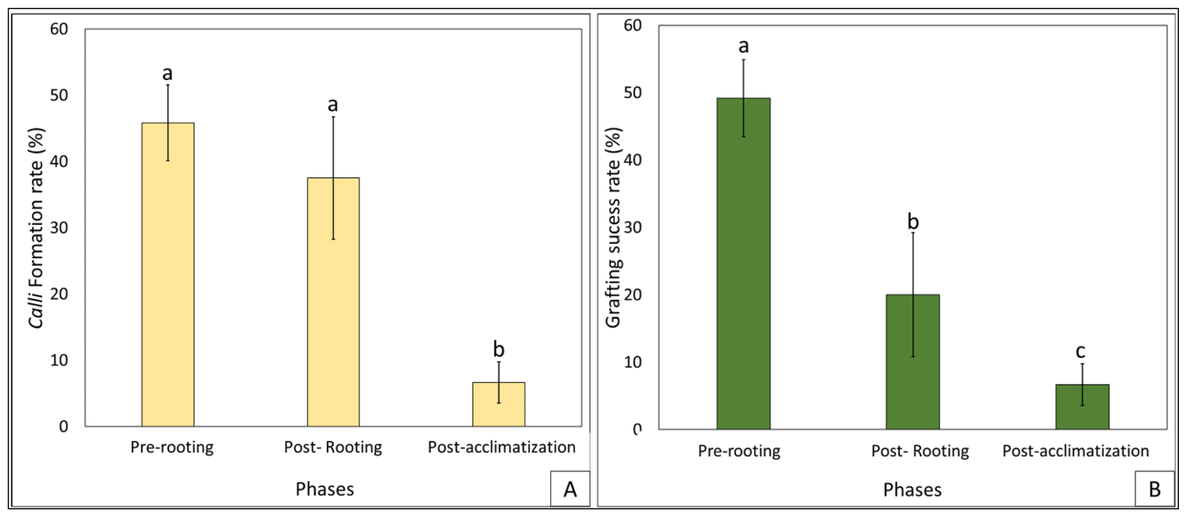

Figure 3 - Differences in the callus formation (A) and in grafting success rates (B), based on the developmental stages of the rootstock, regardless of the grafting method or the cultivar. Different letters represent significant differences for $p \leq 0.05$.

CONCLUSION

Micrografting on rooted rootstocks yielded poor results, likely due to differences in tissue lignification. Optimal outcomes were achieved using *in vitro* scions and rootstocks, independent of cultivar or grafting method, enabling commercial-scale production. However, further optimization is needed, including testing environmental conditions, irrigation during acclimatization, and the optimal scion developmental stage for grafting onto rooted rootstocks.

REFERENCES

- Driver, J. A., & Kuniyuki, A. H. (1984). HortScience, 19(4), 507–509.
- Ribeiro, H., Ribeiro, A., Pires, R., Cruz, J., Cardoso, H., Barroso, J. M., & Peixe, A. (2022). Agronomy, 12(3).