AGRI MAGAZINE
(International E-Magazine for Agricultural Articles)
Volume: 02, Issue: 05 (May, 2025)
Available online at http://www.agrimagazine.in

From Lab to Orchard: A Breakthrough in *in vitro* Establishment of Walnut

Shagun Sharma and *Pankaj Kumar
Department of Biotechnology, Dr. Y.S. Parmar University of Horticulture and Forestry, Solan, Himachal Pradesh, India
***Corresponding Author's email: pksharmabiotech@gmail.com**

Novel medium formulation, "UHF-PKW" has been developed to address the challenges faced in walnut micropropagation, particularly in improving explant survival, reducing browning and enhancing regeneration. Traditional media formulations, have shown limited success, particularly for cultivars like Chandler. The UHF-PKW medium incorporates a precise combination of salts, plant growth regulators and stress-mitigating additives, which collectively enhance explant viability and promote consistent shoot multiplication. This innovative medium formulation represents a significant advancement in the efficiency of walnut tissue culture, offering a reliable and reproducible solution for large-scale propagation. The UHF-PKW medium provides a promising foundation for both research and commercial applications in walnut cultivation.

Introduction

Walnut often referred to as the "king of nuts" that holds a special place in both culinary traditions and commercial agriculture. Rich in nutrients and health benefits, they are in high demand across global markets. Yet, researchers and growers have long grappled with a difficult truth: walnuts are notoriously hard to propagate under laboratory conditions. Unlike other fruit crops such as apples, pears and cherries which respond well to standard tissue culture techniques walnuts exhibit a *recalcitrant behavior*. This means they often resist growth in artificial conditions, leading to poor survival, tissue browning, phenol exudation, microbial contamination and ultimately, low success in regenerating healthy plants. For years, researchers have relied on various culture media such as MS, DKW and WPM to support the growth of walnut plantlets. While these media have shown modest success, they often fall short when applied to walnut, especially the widely cultivated 'Chandler' variety. The results have been inconsistent, the protocols unreliable and the regeneration rates unpromising. Considering this newly formulated medium called "UHF-PKW" aims to enhance *in vitro* viability, growth and multiplication potential by optimizing the balance of macro and micronutrients, plant growth regulators and essential additives. Through systematic assessment of the limitations of existing media, the UHF-PKW formulation not only improves the success rate of *in vitro* establishment but also enhances the overall quality of regenerated plantlets. Globally, scientists like Vahdati et al. have pioneered protocols for the *in vitro* propagation of walnut, setting the foundation for advancements in this field. However, despite these global strides, India still lacks a standardized and effective tissue culture method for walnut, leaving a significant gap in local walnut biotechnology. This gap in reliable propagation has far-reaching consequences. Conventional methods like seed propagation or grafting come with limitations. For trees that require several years to mature and bear fruit, the time lost during this period constitutes a substantial missed opportunity, both for farmers and the walnut industry. Notably, the successful development of this tissue culture method comes at a time when no *in vitro* cultures of Persian walnut (*Juglans regia* L.)

are currently available across India, thereby filling a critical void in the country's walnut propagation.

A new breakthrough in walnut tissue culture

When it comes to growing walnut plantlets in the lab, researchers have tried several medium compositions over the years. The most commonly used one is called the DKW medium, developed by Driver and Kuniyuki (1984). Since then, some researchers have tweaked this formula to try and improve the results. But even with numerous changes, success has been limited. Buds and shoots often fail to survive and those that do, rarely grow well enough to make this method practical for large-scale use. Recognizing these challenges, we came up with a new kind of nutrient-rich medium tailored especially for the Chandler walnut variety i.e., UHF-PKW. This medium contains a unique mix of salts and special ingredients like silver nitrate, casein hydrolysate, activated charcoal, polyvinylpyrrolidone (PVP) and phloroglucinol. These components work together to help the plant tissue stay healthy, reduce browning and less phenol exudation. What makes this development stand out is the impressive success rate. In trials, more than 63% of walnut buds successfully regenerated with this new formulation, a significant improvement over older media, while 76% of embryonic axes germinated. The results have been documented in recent publications by Sharma et al. (2025a) and Sharma et al. (2025b), highlighting the significance of this advancement. Given its significant improvement over existing formulations and reproducible efficacy across trials, this novel medium formulation has also been filed for intellectual property protection under the Indian Patent Office (Application No. 202411082458; Application Date: 28/10/2024). The development of the UHF-PKW medium marks a significant advancement in walnut micropropagation, offering a reliable, reproducible and scalable solution for efficient *in vitro* establishment and shoot multiplication. Looking ahead, the technology will be transferred to stakeholders, including government and private nurseries, tissue culture labs and horticultural research institutions. This will be achieved through training programs, licensing agreements and collaborative demonstrations, ensuring smooth adoption and successful field-level implementation.

Improvements over the existing methods

Regeneration rate: Previous studies on walnut tissue culture have primarily focused on the use of the DKW medium, with modifications aimed at improving explant survival and regeneration rates. However, these existing protocols often face limitations, including inconsistent survival rates, poor shoot multiplication and challenges with explant browning (Tarinejad 2013). While some advancements, such as the inclusion of Plant Preservative Mixtures (PPM) or additional growth regulators, have shown modest improvements, they have not delivered consistent results, particularly for the Chandler cultivar of *J. regia*. In contrast, the novel UHF-PKW medium developed offers significant improvements in both explant survival and shoot multiplication. By optimizing nutrient compositions and incorporating specialized additives, we have observed a marked enhancement in regeneration rates and overall plant health.

Multiplication: The efficiency of shoot multiplication from explants in walnut tissue culture has long been a challenge, as confirmed by earlier studies, such as by Dirlik et al. (2022), which reported limited shoot multiplication rates with traditional protocols. These methods often result in a low number of shoots per explant, making large-scale propagation difficult. In contrast, our optimized medium formulation demonstrates a significant improvement in shoot multiplication. By carefully balancing growth regulators and incorporating key additives, we have been able to enhance the multiplication rate, yielding more shoots per explant compared to previous methods. This advancement is crucial for scaling up walnut propagation, offering a more effective solution for tissue culture-based walnut production.

Acclimatization: For the first time, successful acclimatization was achieved by directly transferring non-rooted regenerated shoots derived from callus to a soil-based medium comprising cocopeat, perlite and vermiculite in a 1:1:1 ratio. This approach has proven to be

both effective and efficient, offering an alternative to traditional *in vitro* rooting followed by *ex vitro* hardening, as seen in previous studies by Vahdati et al. (2004) and Ribeiro et al. (2022). Our method simplifies the process and enables the establishment of plantlets under greenhouse conditions, enhancing the potential for practical, large-scale application.

Importance of innovation

Advancing walnut micropropagation: Walnut cultivation, particularly of elite cultivars like Chandler, has been constrained by the challenges of conventional propagation methods and poor *in vitro* response. The development of an improved *in vitro* protocol using a novel medium addresses these long-standing limitations, enabling higher regeneration, multiplication and survival rates. This advancement holds significant potential for enhancing the efficiency and reliability of commercial walnut plant production.

Bridging the demand-supply gap: The domestic and global demand for high-quality walnuts continues to rise. However, walnut propagation remains a bottleneck, limiting supply and productivity. The implementation of this efficient micropropagation protocol can help address this gap by providing a scalable solution for mass production of disease-free, true-to-type walnut plants, thereby strengthening local production systems and reducing reliance on imports.

Applicability across varieties: While the protocol was optimized for *Juglans regia* cv. Chandler, its efficacy has also been validated in other walnut varieties including Vlach rootstock, Lara, Howard, Chico, Tulare and other indigenous cultivars. This wide applicability broadens the scope of the method, making it suitable for use across diverse agro-climatic zones and breeding programs.

Support for quality certification systems: The consistency and reproducibility of this protocol aligns well with the objectives of the National Certification System for Tissue Culture Raised Plants (NCS-TCP). Adoption of this method can support certified nurseries in maintaining high quality standards for tissue culture-raised walnut plants, thereby increasing assurance among growers and promoting uniform orchard establishment.

Conclusion

The development of this new medium UHF-PKW presents a breakthrough in the field of walnut micropropagation by introducing a novel medium formulation tailored specifically for the *in vitro* establishment and multiplication of the Chandler cultivar. Unlike earlier protocols, which often fell short in terms of reproducibility, regeneration efficiency and acclimatization success, the newly developed method offers a simplified and dependable solution to these persistent challenges. The medium's compatibility with other walnut genotypes further highlights its broad utility and commercial potential.

References

1. Dirlik, C., Kandemir, H., Cetin, N., Sen, S., Guler, B. and Gurel, A. (2022). Effects of different culture media compositions on in vitro micropropagation from paradox walnut rootstock nodes. *Gazi University Journal of Science Part A: Engineering and Innovation*, **9**(4): 500-515.
2. Driver, J.A. and Kuniyuki, A.H. (1984). *In vitro* propagation of Paradox walnut rootstock. *HortScience*, **19**(1): 507-509.
3. Ribeiro, H., Ribeiro, A., Pires, R., Cruz, J., Cardoso, H., Barroso, J.M. and Peixe, A. 2022. *Ex vitro* rooting and simultaneous micrografting of the walnut hybrid rootstock 'Paradox' (*Juglans hindsi*×*Juglans regia*) cv.'Vlach'. *Agronomy*, **12**(1): 595.
4. Sharma, S., Sharma, S. and Kumar, P. (2025a). *In vitro* establishment of walnut (*Juglans regia* L.) from the embryonic axis with customized nutrient media and synergy of growth regulators. *South African Journal of Botany*, **177**: 598-603.
5. Sharma, S., Sharma, S., Kumar, P., Verma, P., Sharma, R. and Sharma, N.C. (2025b). Overcoming Challenges in Walnut (*Juglans regia* L.) Micropropagation: A

Comprehensive Approach to Optimizing Explant Selection, Sterilization, and Nutrient Media. *Applied Fruit Science*, **67**(1): 1-13.

6. Tarinejad, A. (2013). Effects of disinfectants and antibiotics on contamination during propagation of walnut (*Juglans regia* L.). *Research on Crops*, **14**: 219-225.
7. Vahdati, K., Jariteh, M., Niknam, V., Mirmasoumi, M. and Ebrahimzadeh, H. (2004). Somatic embryogenesis and embryo maturation in persian walnut. In *V International Walnut Symposium*, **705**: 199-205.