

REVIEW

Open Access



Nodal culture for efficient regeneration and CRISPR/Cas-based genome editing in recalcitrant horticultural crops

Himanshu Pandey^{1†}, Varucha Misra^{2*†}, Avinash Sharma^{3*†}, Basistha Chatterjee³, Monoj Sutradhar⁴, Rajeev Kumar⁵, Punabati Heisnam⁶, V. S. Devadas⁷, Ashutosh Kumar Mall², Nimisha Tehri⁸ and Amit Vashishth⁹

Abstract

Nodal culture is a powerful plant tissue culture technique addressing critical challenges such as desiccation, microbial contamination, and the limited viability of explants, particularly in recalcitrant horticultural crops like *Garcinia mangostana*, *Artocarpus heterophyllus*, *Cucumis melo*, *Citrus limon*, *Kinnow mandarin*, and *Coffea arabica*. This method utilizes sterilized immature nodal explants, with regeneration induced through the precise application of growth regulators, primarily auxins and cytokinins, to media such as Driver-Kuniyuki (DKW), Woody Plant Media (WPM), and Murashige and Skoog (MS) under controlled conditions. These regulators significantly enhance both shoot and root regeneration, thus reducing the generation time for difficult-to-regenerate species. Reactive oxygen species (ROS) play a pivotal role in regulating cell division and hormone signaling during regeneration. Additionally, transcription factors such as wound-induced dedifferentiation 1 (WIND1), WUSCHEL (WUS), Enhancer of Shoot Regeneration 1 (ESR1), Cup-shaped Cotyledon 1 and 2 (CUC1, CUC2), and Lateral Organ Boundaries Domain 16 (LBD16) are integral to callus induction and organogenesis. Genetic variation observed in regenerated populations reflects the complexity of these regulatory networks and underscores the need for further investigation. Notably, nodal culture provides a promising alternative to conventional tissue culture methods, particularly in facilitating CRISPR/Cas9-mediated genetic modifications in recalcitrant crops. This technique enhances the efficient regeneration of transgenic horticultural crops, overcoming significant barriers to transformation. Future research should focus on refining nodal culture protocols across a broader spectrum of horticultural species, improving gene editing efficiency, and integrating this approach with advanced breeding technologies for targeted trait development and sustainable crop improvement.

Keywords Nodal culture, Recalcitrant horticultural plant, Genetic transformation, Regeneration, Speed breeding, Abiotic/Biotic stress

Introduction

Recalcitrant crops, are distinguished by their high water content in seeds, typically ranging from 30–70%. These seeds are highly sensitive to low temperatures, with germination and other biological processes being inhibited below 10 °C in cold climates and at –20 °C under freezing conditions (Barbedo et al. 2013). Recalcitrant horticultural crops, such as *Mangifera indica*, *Garcinia mangostana*, *Cymbopetalum baillonii*, *Areca catechu*, *Calamus* spp., *Nymphaea caerulea*, *Viola*, *Pentaclethra*, and various horticultural trees, frequently encounter abiotic stresses, including cold,

[†]Himanshu Pandey, Varucha Misra and Avinash Sharma contributions equally to this work.

*Correspondence:

Varucha Misra
misra.varucha@gmail.com
Avinash Sharma
avinashcau@gmail.com

Full list of author information is available at the end of the article

freezing, heat, and waterlogging (Sujith 2015). Exposure to these stresses results in the rapid loss of seed viability due to their susceptibility to desiccation within 3–4 days (Pritchard et al. 2022). These crops play vital roles in food security, pharmaceutical applications, ecological balance, and biogeochemical cycle maintenance due to their biochemical diversity and environmental functions, such as carbon footprint reduction and carbon sequestration (Jain et al. 2023; Bhattacharyya et al. 2015; Lal et al. 2018).

However, propagating and improving recalcitrant horticultural crops presents significant challenges, primarily due to their limited totipotency during *in vitro* regeneration. This restriction hampers their ability to undergo direct or indirect regeneration, even when advanced tissue culture techniques such as cell culture, suspension culture, seed culture, embryo culture, endosperm culture, ovary culture, ovule culture, and protoplast fusion or somatic hybridization are applied under controlled conditions (Patel et al. 2022). Additionally, the high water content in explants such as seeds, root cells, shoot cells, leaf cells, ovaries, ovules, anthers, axillary buds, root tips, and shoot tips significantly impairs nutrient uptake and cell division (Ricroch et al. 2024; Berjak and Pammenter 2004; Mahmud 2012), inhibiting regeneration in controlled environments, often resulting in regeneration failure under cold or freezing conditions (Majumder et al. 2023). This excess water also interferes with key factors, including media composition, growth regulator concentrations, light intensity, temperature, and humidity, reducing regeneration efficiency and promoting media solidification (Fernandez et al. 2023). Moreover, water retention disrupts the cellular, molecular, and biochemical processes necessary for regeneration, particularly in explants rich in parenchymatous tissue such as roots, shoots, leaves, ovaries, ovules, anthers, axillary buds, and

endosperm, where it leads to slowed cell division and regeneration (Fig. 1) (Fernandez et al. 2023).

Nodal culture is a sophisticated and reliable plant tissue culture technique with significant potential for facilitating both direct and indirect regeneration in explants from recalcitrant horticultural crops under controlled environmental conditions (Bennur et al. 2005). This method utilizes immature node explants, effectively addressing challenges such as desiccation, limited storage viability, microbial contamination, and the short lifespan of recalcitrant plant materials (Perera et al. 2015). Immature nodes from these crops contain abundant intercalary and apical meristematic cells, which are characterized by high mitotic activity and enhanced totipotency. Additionally, these nodes retain less water compared to other explants, making them more conducive to regeneration (Lee 2023). Leveraging this regenerative potential, shoot and root meristems serve as highly organized and dynamic centers of cellular proliferation, consisting of embryonic-like cells that continuously generate both aerial and subterranean plant organs through mitosis (Amin et al. 2002). Upon germination, a specific subset of stem cells within these meristems is activated to initiate the formation of lateral organ primordia, while a central population of undifferentiated pluripotent cells maintains meristematic identity and ensures long-term self-renewal (Kwiatkowska 2008). Unlike floral meristems, which are transient and determinate, the shoot apical meristem retains stem cell activity throughout the plant's lifespan (Chang et al. 2020). Pre-formed meristem protocols are designed to exploit the inherent developmental plasticity of various meristem types—apical, axillary, and adventitious—from the earliest stages of seedling emergence to senescence (Belanger et al. 2024). When applied under precisely controlled *in vitro* conditions, immature nodes respond positively

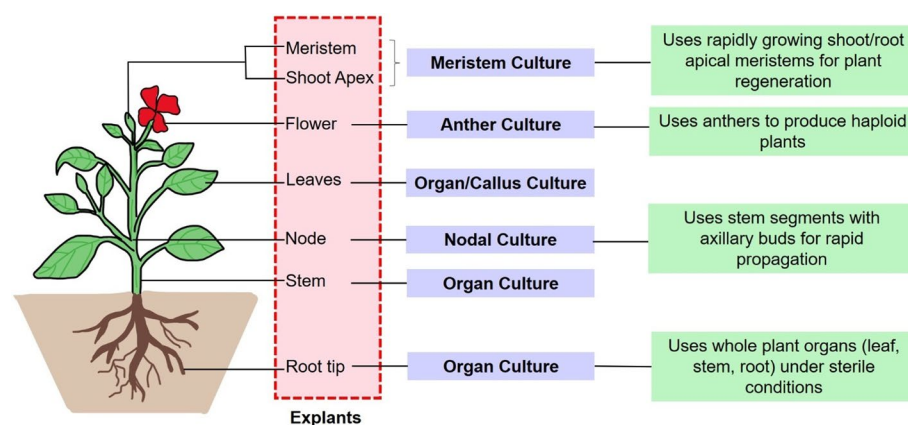


Fig. 1 Nodal culture and nodal explants in comparison with other explant types and tissue culture methods for plant regeneration. The figure compares nodal culture, which uses nodal explants for direct shoot proliferation from axillary buds, with other tissue culture methods, including shoot tip, leaf, root, and callus cultures. Nodal culture provides superior genetic stability, efficient clonal propagation, and minimal somaclonal variation compared to callus-based techniques

to key factors such as media composition, growth regulator concentrations, light intensity and quality, and temperature, all of which significantly influence regeneration success (Sehgal and Joshi 2022). Furthermore, stress-induced accumulation of reactive oxygen species (ROS) and changes in endogenous phytohormone levels modulate cellular reprogramming and morphogenic responses, ultimately affecting the regeneration outcome. Integrating nodal culture with meristem-based strategies offers a robust platform to overcome recalcitrance and improve regeneration efficiency in challenging plant systems.

Given the limited research on regeneration in recalcitrant horticultural crops, it is crucial to develop strategies that enhance both direct and indirect regeneration processes under controlled environmental conditions. Equally important is integrating advanced molecular techniques, such as speed breeding and CRISPR/Cas-mediated genome editing, into nodal culture. These integrated approaches help overcome physiological barriers, improve regeneration efficiency, facilitate genetic transformation, and enable the development of biotic and abiotic stress-tolerant recalcitrant plant varieties (Akhtar et al. 2022). Despite these advancements, significant gaps remain in understanding regeneration protocols across different recalcitrant species, as well as in elucidating the biochemical and genetic regulatory networks that govern successful regeneration. This review aims to advance both theoretical insights and practical applications in the propagation and genetic improvement of recalcitrant horticultural crops.

Nodal culture protocol and response in recalcitrant horticultural crops

Recalcitrant crops can be successfully regenerated from nodal explants using specialized media and growth regulators under controlled environmental conditions. Immature nodal explants, typically 1–2 cm in length, are initially cleaned with a liquid detergent (Tween 20) and thoroughly rinsed with distilled water for 20 min. Tween 20 acts as a wetting agent, facilitating the even distribution of disinfectants and aiding in the removal of oils and organic materials from the explants' surface, thereby reducing contamination risks during tissue culture. Following this detergent treatment, the explants are immersed in a fungicide-bactericide solution containing carbendazim and streptomycin for 20 min, after which they are washed 4–5 times with distilled water to eliminate any residual chemicals. Carbendazim, a broad-spectrum fungicide, inhibits fungal cell division and growth by disrupting microtubule formation during mitosis (Shields et al. 1984), while streptomycin, a broad-spectrum antibiotic, effectively controls bacterial species, including *Ralstonia solanacearum*, which poses

a significant threat to plant health. The combination of carbendazim and streptomycin provides a synergistic defense against microbial contamination in nodal cultures. For chemical sterilization, the explants are immersed in 70% ethanol for 5 min, followed by treatment with 1% or 0.8% sodium hypochlorite for 20 min (Boboc et al. 2022). Ethanol serves as a rapid surface disinfectant, while sodium hypochlorite ensures deeper, more comprehensive sterilization. This sequential disinfection process effectively minimizes contamination and enhances the success rate of tissue culture procedures. The sterilized nodal explants are then inoculated onto Murashige and Skoog (MS) or Driver-Kuniyuki (DKW) media, supplemented with 0.01–2 mg/L auxin, 0.4–4 mg/L cytokinin, or a combination of both. The cultures are maintained under a 16-h light/8-h dark photoperiod at 25 ± 2 °C. Shoot regeneration typically occurs within 4–8 weeks, depending on the media composition and growth regulator concentrations. The regenerated shoots are then transferred to root induction media, either half-strength Woody Plant Media (WPM) or full-strength DKW media, containing 0.1–2 mg/L of auxin. Root development is observed within 4 weeks under the same environmental conditions. The regenerated plantlets are then acclimatized in pots containing a mixture of peat and perlite (2:1) under >85% relative humidity in the growth chamber. After initial acclimatization, the plantlets are gradually transferred to an open plastic tunnel or greenhouse for 15–30 d to complete the hardening process. This transitional phase is essential for adapting the crops to external conditions, ultimately improving survival and establishment rates upon their transfer to the field (Fig. 2) (Pérez-Tornero et al. 2010).

Recalcitrant horticultural crops exhibit both direct and indirect regeneration, which are influenced by factors such as the chemical composition of the growth medium, plant growth regulators (PRGs), and controlled environmental conditions (Long et al. 2022). Several recalcitrant species, including *Garcinia mangostana*, *Ixora coccinea*, *Ixora fulgens*, *Citrus limon* L., *Shorea robusta*, *Avicennia marina*, *Citrus limon* 'Kaghzi Kalan', *Coffea arabica*, *Artocarpus heterophyllus*, *Calophyllum brasiliense*, *Cucumis melo*, *Theobroma cacao*, *Piper aduncum*, *Piper hispidinervum*, and Kinnow mandarin, have been successfully regenerated, though their regeneration responses vary depending on timeframes and controlled environmental conditions (Table 1) (Ochatt 2024). Specific media compositions and PRGs have demonstrated significant potential in facilitating both direct and indirect regeneration via nodal culture (Mosoh et al. 2024). However, further research is necessary to explore regeneration in additional recalcitrant species by refining media formulations and phytohormonal

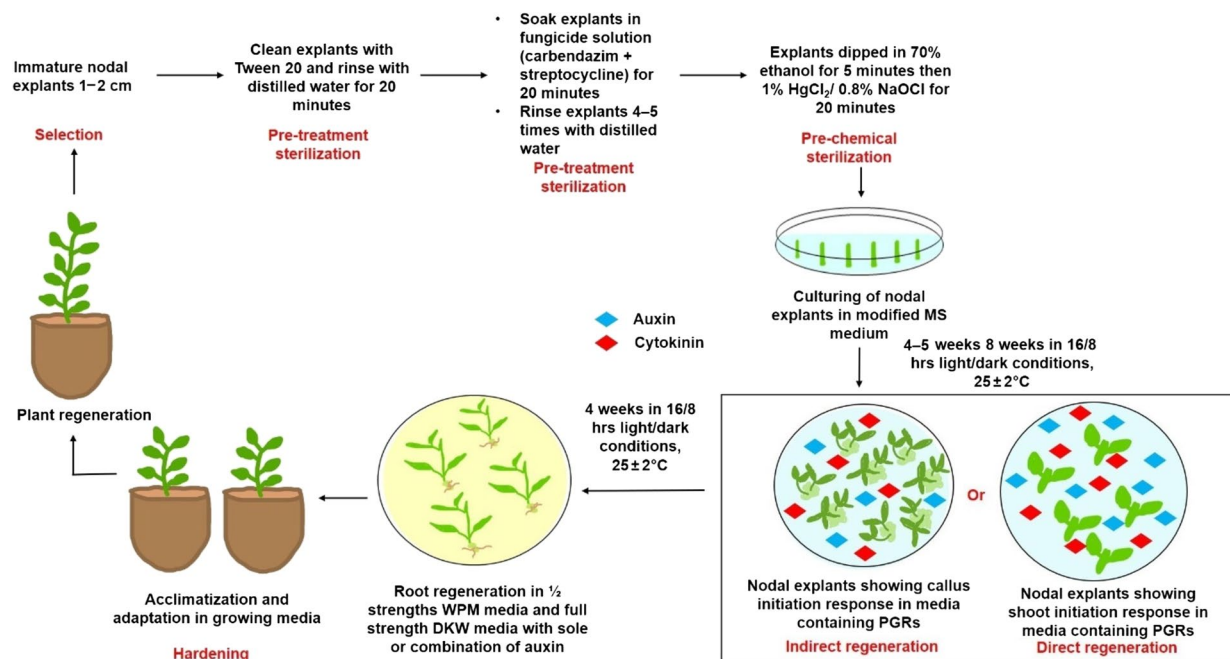


Fig. 2 Schematic diagram of the protocol for direct and indirect plant regeneration of recalcitrant horticultural crops via nodal culture. Direct shoot regeneration occurs from nodal meristematic regions without callus formation, while indirect regeneration involves callus initiation prior to shoot differentiation. PGRs, plant growth regulators; WPM, Woody Plant Media; DKW, Driver-Kuniyuki; MS, Murashige and Skoog

interventions. Given its efficiency, nodal culture offers substantial contributions to advancing the development of biotic and abiotic stress-resistant plant populations (Pumisutapon et al. 2012). Puijalón (2008) investigated the regeneration capacity of rhizome explants under various abiotic stresses, including exposure to warm water, hot air, cold water, and sodium chloride, revealing the beneficial effects of these treatments. Similarly, Jose and Thomas (2015) demonstrated the positive impact of abiotic stresses on shoot regeneration in *Curcuma caesia* rhizome segments under in vitro conditions. Advances in gene profiling have identified molecular markers linked to plant regeneration. Notably, RAP2.6L (At5g13330), a member of the ERF/APETALA2 transcription factor (TF) family, has been identified as a key regulator during the early stages of shoot induction. RAP2.6L is thought to regulate the expression of multiple genes essential for shoot regeneration (Che et al. 2006). Ravindran (2007) observed significant variability in the regeneration capacity and stress tolerance of different plant cultivars. Moreover, the application of nanoparticles (NPs) has shown promise in enhancing tissue culture outcomes. NPs influence callus induction, organogenesis, somatic embryogenesis, somaclonal variation, genetic transformation, and secondary metabolite production. Their antimicrobial efficacy depends on factors such as particle size, type, distribution, and dimensions (Kim et al. 2017). However, the precise mechanisms through which NPs modulate regeneration remain poorly

understood and warrant further investigation. Regenerated horticultural crops from these protocols can be integrated into speed breeding programs and CRISPR/Cas-based genome editing techniques within controlled environments.

Biochemical mechanisms underlying plant regeneration via nodal culture

Immature nodal explants synthesize a wide array of biomolecules, including alkaloids, terpenoids, and both primary and secondary metabolites, which are critical for shoot and root regeneration. These compounds serve not only as defense agents but also play active roles in regulating regenerative processes. Alkaloids and terpenoids, acting as signaling molecules, influence gene expression and activate pathways that enhance meristematic activity, thus facilitating the initiation of new shoots and roots. Specific terpenoids, for example, modulate the activity of plant hormones such as cytokinins and auxins, which are essential for cell division, differentiation, and organogenesis. Alkaloids also help modulate stress responses, thereby enhancing the explant's adaptability to in vitro conditions and improving regeneration efficiency. Primary metabolites, such as sugars and amino acids, provide the necessary energy and building blocks for tissue formation and cellular differentiation. Concurrently, secondary metabolites, synthesized in response to wounding or culture stress, create a protective environment that

Table 1 Regeneration of recalcitrant horticultural crops in nodal culture

Crops	Media		Callus	Callus Induction (days)	Shoot Regeneration (days)	Root Regeneration (days)	References
	Shoot	Root					
<i>Cucumis melo</i> L	MS + 1 mg/L BAP, MS + 1.5 mg/L BAP + 0.1 mg/L GA ₃	1/2MS + 0.5–3 mg/L IBA, MS + 0.5–2 mg/L NAA	-	-	15–21	15–30	Kim et al (2017)
<i>Artocarpus heterophyllus</i>	MS + 3.5 mg/L BAP, MS + 3.5 mg/L BAP + 1.5 mg/L kinetin	1/2MS + 1.2 mg/L IBA	-	-	28	28	Parvin et al. (2013)
<i>Ixora fulgens</i>	MS + 0.5 mg/L BA + 0.1 mg/L NAA	1/2 MS + 0.1–0.5 mg/L NAA/ IBA/IAA	MS + 3 mg/L 2,4-D, 1 mg/L BA + 1 mg/L NAA, 0.2 mg/L BA + 1 mg/L IBA	-	49–70	8–30	Azam et al. (2009)
<i>Artocarpus heterophyllus</i>	MS + 2.5 mg/L BA + 0.5 mg/L NAA	1/2MS + 1 mg/L IBA + 1 mg/L NAA	-	-	-	21	Roy et al. (2000)
<i>Ixora species</i>	MS + 1 mg/L BA, MS + 0.2 mg/L NAA	1/2 MS + 0.1–0.5 mg/L NAA/ IBA/IAA	MS + 2 mg/L 2,4-D	-	56	25	Sivakumar et al. (2020)
Kinnow mandarin	MS + 2.9 mg/L BAP + 0.06 mg/L NAA	1/2 WPM + IBA + IAA	-	-	28–35	-	Singh et al. (2018)
<i>Shorea robusta</i>	WPM + 1 mg/L BA + 0.5 mg/L NAA	WPM + 0.5 mg/L IBA	-	-	28	21	Singh et al. (2014)
<i>Artocarpus heterophyllus</i>	MS + 2 mg/L BA, MS + 1–2 mg/L BA + 0.35 mg/L GA ₃ , MS + 3 mg/L BA + 0.35 mg/L GA ₃ , MS + 2.5 mg/L BA + 0.35 mg/L GA ₃ , modified MS media + 2 mg/L BA, Gamborg B5 media + 2 mg/L BA, WPM + 2 mg/L BA	1/2 MS + 1 or 3 mg/L NAA, 1/2 MS + 0.5 or 1 mg/L IBA	-	-	13–21	98–112	Av and Karishma (2024)
Seedless limon (<i>Citrus limon</i> Kaghzi Kalan)	MS + 0.5 mg/L kinetin + 1 mg/L BA, MS + 1 mg/L kinetin + 0.5 mg/L IBA, MS + 0.25 mg/L BA + 1 mg/L kinetin + 1 mg/L IBA	MS + without growth regulators	-	-	56	60	Goswami et al. (2013)
Lemon (<i>Citrus limon</i> L)	MS + 1 mg/L BA	DKW + 1 mg/L IBA, 3 mg/L IBA, DKW + 1 mg/L IAA	-	-	56	28	Pérez-Tornero et al. (2010)
<i>Cucumis melo</i> L	MS + 8 mg/L BA, MS + 0.5 mg/L BA	1/2 MS + without growth regulators	MS + 8 mg/L BA, MS + 2 mg/L NAA	42	21	28	Rupp et al. (1999)
<i>Avicennia marina</i>	MS + 1 mg/L kinetin + 0.5 mg/L BA, MS + 2 mg/L kinetin + 0.01 mg/L IBA + 0.5 mg/L BA	MS + 0.5 mg/L IBA, MS + 0.5 mg/L NAA	-	-	28	28	Kareem et al. (2015)

Table 1 (continued)

Crops	Media		Root	Callus	Callus Induction (days)	Shoot Regeneration (days)	Root Regeneration (days)	References
	Shoot							
<i>Coffea arabica</i> 'MCH2'	MS + 10 mg/L BA, MS + 25 mg/L adenine hemisulfate		1/2 MS + 1.5 mg/L IBA	-	-	28	70	Gebremariam (2017)
<i>Persea americana</i> Mill	WPM + 0.1 mg/L metatopolin + 0.1 mg/L GA ₃ , MS + 0.65 mg/L BAP, MS + 2 mg/L BAP + 0.5 mg/L IBA		WPM + 3.5 mg/L IBA, MS macronutrients, WPM + 0.5 mg/L IBA + 1 g/l activated charcoal + 0.4 g/l ascorbic acid	-	6–17	13	-	Hiti-Bandaralage et al. (2017)
<i>Theobroma cacao</i> L	1/2 DKW + 10 g/l glucose + 0.3 g/l KNO ₃ + 1.75 g phytagel + 2.5 µM arginine + 2.5 µM leucine + 2.5 µM tryptophane + 0.01–0.4 µM thidiazuron		Media + 15 µM IBA	-	-	42	28–42	Traore et al. (2003)
<i>Piper aduncum</i>	MS + 1–3 mg/L BAP + 0.05 mg/L NAA, WPM + 1–3 mg/L BAP + 0.05 mg/L NAA		-	MS + 1–3 mg/L BAP + 0.05 mg/L NAA, WPM + 1–3 mg/L BAP + 0.05 mg/L NAA	28	28	-	da Silva et al. (2012)
<i>Piper hispidinervum</i>	MS + 1–3 mg/L BAP + 0.05 mg/L NAA, WPM + 1–3 mg/L BAP + 0.05 mg/L NAA		-	MS + 1–3 mg/L BAP + 0.05 mg/L NAA, WPM + 1–3 mg/L BAP + 0.05 mg/L NAA	28	28	-	da Silva et al. (2012)
<i>Coffea arabica</i> 'mundo novo'	MS + 5–25 µM/L BA		-	-	-	-	10	Ramos and Almeida (2005)
<i>Calophyllum brasiliense</i>	WPM + 4.4 or 4.8 µM BAP		WPM + 10 or 20 µM IBA, WPM + 10 or 20 µM NAA	-	-	30–90	60	Silveira et al. (2016)
<i>Hevea brasiliensis</i> Mull. Arg	MS + 2 g/L activated charcoal + 1 mg/L silver nitrate + 2 mg/L GA ₃ + 5 mg/L kinetin		-	-	-	28	-	Antwi-Wiredu et al., (2018)
<i>Persea americana</i> Mill	WPM + 1.5 mg/L BAP, MS + 1.5 mg/L BAP		WPM + 1 mg/L IBA, MS + 1 mg/L BAP	-	-	35–42	42	Sholi and Qasrawi (2022)
<i>Gracilaria mangostana</i>	WPM + 1 mg/L BAP		-	MS + 5 mg/L BAP	6–17	13	-	Isda et al. (2019)

MS, Murashige and Skoog; WPM, Woody Plant Media; DKW, Driver-Kuniyuki; 2,4-D, 2,4 dichlorophenoxy acetic acid; NAA, naphthalene acetic acid; IBA, indole butyric acid; IAA, indole acetic acid; BAP, benzyl adenopurine; BA, benzyl adenine; GA₃, Gibberellic acid

mitigates oxidative damage and supports tissue viability. Optimizing culture conditions—such as media composition, temperature, photoperiod, and plant growth regulators (PGRs) concentration—can significantly boost secondary metabolite production (Isah et al. 2018). The careful selection of media, supplemented with essential macro- and micro-nutrients, along with hormones like auxins, cytokinins, and gibberellins, further promotes metabolite synthesis (Cardoso et al. 2009). Nutrient metabolism, plant hormone signaling, and ROS regulation are pivotal in modulating regeneration processes in these crops (Faizan et al. 2023; Elhiti and Stasolla 2014). Plant tissue culture techniques, such as callus induction, cell suspension, and shoot cultures, are commonly used to produce biomass for in vitro secondary metabolite synthesis. Among these, immature nodal explants offer distinct advantages for regeneration due to their responsiveness to specific culture stimuli (Guerriero et al. 2018). Under controlled environmental conditions, ROS stimulate key physiological and molecular processes, including programmed cell death, plant hormone signaling, and cell division, all of which contribute to shoot induction (Bidabadi and Jain 2020). While ROS are traditionally associated with stress, in tissue culture, they act as positive regulators of apoptosis, metabolic activation, and signal transduction, essential for regeneration (Luo et al. 2019). However, further studies are required to clarify the gene regulatory networks involved in ROS-mediated regeneration and their specific role in recalcitrant plant species (Fig. 3).

Lastly, the biosynthetic pathways within nodal explants are responsible for producing regulatory molecules and assimilates essential for plant research (Zhumanova et al. 2024). Future research should focus on characterizing

these enzymatic pathways, identifying key precursor molecules and end products, and investigating the expression of genes involved in these biosynthetic routes, particularly in recalcitrant species. Such insights could significantly advance our understanding of regeneration mechanisms in difficult-to-propagate crops.

Cellular mechanism of plant regeneration

The anatomical structures of plant explants vary significantly across species. For instance, leaf explants from *Arabidopsis thaliana* consist of procambium and vascular parenchyma cells, while cereal crop leaf explants contain bundle sheath cells and immature vascular cells. Root explants also display species-specific differences: in *Arabidopsis*, the root is primarily composed of xylem-pole pericycle cells, whereas in cereal crops, the roots are predominantly made up of phloem-pole pericycle cells (Hu et al. 2017). In leaf explants, two types of vascular bundle sheaths form: an inner and an outer sheath. The inner sheath exhibits active cell division, while the outer sheath contributes to callus formation. In root explants, the phloem-pole pericycle initiates callus formation, with lateral roots facilitating organ development. Both root tip and lateral root explants rely on phloem-pole pericycle cells to activate callus formation (Zeng et al. 2016). These regenerative processes are regulated by specific TFs that control the cell cycle, founder cell initiation, and callus development. For example, WUSCHEL-RELATED HOMEODOMAIN 11 and 12 (WOX11/12) drive founder cell division in both root and leaf explants, while WOX5 further promotes the conversion of founder cells into undifferentiated parenchymatous cells, facilitating callus or wound tissue formation (Liu et al. 2014). The internal structures of explants also influence cell division,

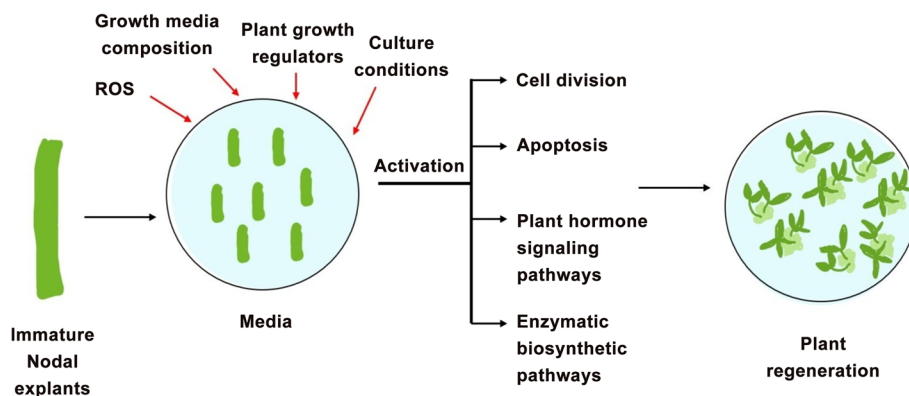


Fig. 3 Biochemical mechanisms of plant regeneration in nodal culture. The figure illustrates key hormonal and biochemical responses in nodal explants during plant regeneration. Cytokinin-rich media stimulate meristem activation by inducing genes like WUS, STM, and CYCD, while ROS like H_2O_2 acts as signaling molecule for cell reprogramming. Enhanced activity of antioxidant enzymes (SOD, CAT, APX) helps maintain redox balance. Secondary metabolites modulate hormone levels and tissue responsiveness, collectively promoting successful shoot initiation and plantlet development. ROS, relative oxygen species

callus formation, and organogenesis (Zai and Xu 2021). In nodal explants, specific tissues likely contribute similarly to these regenerative processes. TFs that regulate cell differentiation, de-differentiation, and transformation play a pivotal role in this context (Fambrini et al. 2022). However, further research is required to identify the internal cellular components in nodal explants responsible for these activities, including the modulation of TF expression (Fig. 4).

Mechanism of gene regulation in recalcitrant horticultural crops regeneration

Organized culture media and crop growth regulators, particularly auxins and cytokinins, are essential for promoting plant regeneration via nodal culture (Pasternak and Steinmacher 2024). The wounding response at the nodal explant site triggers a localized accumulation of auxin and cytokinin, two critical phytohormones that initiate cell dedifferentiation and either promote callus formation or direct organogenesis. Immature nodal explants often respond to callus induction when treated with auxin (2,4-D) and cytokinin. Auxins, through the regulation of biosynthesis genes (e.g. YUCCA family), auxin transport proteins (e.g. PIN), and Auxin Response Factors (ARFs), initiate somatic embryogenesis, callus formation, and root organogenesis. Cytokinins promote shoot induction by modulating TFs such as WUSCHEL (WUS), partly through the activation of type A and type B *Arabidopsis* response regulators. Specific genes and TFs are essential for the auxin response to 2,4-D, facilitating callus formation (Fan et al. 2022). Notably, members of the tryptophan aminotransferase family (TAA/YUCCA/YUC, YUCCA1, YUC1, YUC4) and N-acetylcysteine (NAC), no apical meristem (NAM), and *Arabidopsis* Thaliana Activation Factor 1 and 2 (ATAF1, ATAF2) influence auxin synthesis and signaling (Yang et al. 2023). Additionally, TFs like wound-induced dedifferentiation 1 (WIND1) and histone modifications, including H3K9/14 acetylation and H3K27 methylation, regulate callus formation and wound response in immature nodal explants (Li et al. 2024). The auxin-to-cytokinin ratio is critical for stimulating both root and shoot regeneration from immature nodal explants (Hill and Schaller 2013). Understanding these signaling pathways, particularly those influenced by phytohormones, is central to genetic engineering efforts aimed at improving the regenerative potential of explants in tissue culture. Histidine-containing phosphotransfer (HPT) proteins, critical mediators of phosphate signaling, regulate cytokinin-responsive genes (To et al. 2014). Upregulating genes involved in cytokinin regulation enhances cytokinin sensitivity during the multiplication phase, offering a strategy to overcome recalcitrance in *in vitro* plant regeneration.

High auxin concentrations favor root formation, while elevated cytokinin levels promote shoot regeneration (Mazri et al. 2022). Key regulatory genes and TFs such as histidine protein kinases (AHKs) and histidine phosphotransfer proteins (AHPs) activate the cytokinin signaling pathway (Hoang et al. 2021). TFs like WUS, Enhancer of Shoot Regeneration 1 and 2 (ESR1, ESR2), and Cup-shaped Cotyledon 1 and 2 (CUC1, CUC2) regulate shoot regeneration from immature nodal explants (Rasheed et al. 2024). Furthermore, TFs such as Lateral Organ Boundaries Domain 16 and 29 (LBD16, LBD29) and WOX5 influence auxin-regulated root development, with WOX11, WOX12, LBD16, LBD29, WOX5, and ARFs playing vital roles in root generation (Ikeuchi et al. 2019). Several regulatory genes, including KNOTTED1 (KN1), Shoot Meristemless (STM), WUS, and CLAVATA 1–3 (CLV1–3), play pivotal roles in shoot regeneration by coordinating cytokinin receptor activation, cell cycle progression, and shoot meristem formation (Zhang and Lemuax 2004; Riou-Khamlichi et al. 1999; Kareem et al. 2015). During embryogenesis, TFs like CUC1 and CUC2 are essential for regenerating shoot meristems, activated by PLETHORA TFs (PLT3, PLT5, and PLT7) (Kareem et al. 2015). Wounding induces the expression of key genes, such as PLT1, PLT2, CUC1, CUC2, and WIND1–4, which facilitate the acquisition of pluripotency at the wound site (Gordon et al. 2007; Motte et al. 2014). Upon transfer to cytokinin-enriched shoot induction media, the regulation of WUS expression significantly enhances shoot regeneration (Chatfield et al. 2013). Furthermore, TFs such as ESR1 and ESR2 promote embryogenesis and shoot formation by upregulating CUC1 in response to cytokinin-rich conditions (Kirch et al. 2003).

Root regeneration, particularly in auxin-rich environments, is regulated by WOX11 and WOX12, which activate downstream genes such as LBD16 and LBD29, and subsequently WOX5 (Ikeuchi et al. 2016; Liu et al. 2014). The LBD and WOX5 families also play pivotal roles in lateral root development (Goh et al. 2012). Specific ARFs regulate WOX11 expression during root regeneration from leaf explants. Additionally, WIND1, a member of the APETALA2/ethylene-responsive element-binding family, is essential for promoting regenerative competence (Iwase et al. 2011). While WIND1 does not directly induce somatic embryo formation, it is critical for callus induction during indirect somatic embryogenesis, acting upstream of LEAFY COTYLEDON 2 (LEC2) (Iwase et al. 2015). Together, these TFs and regulatory genes coordinate both direct and indirect plant regeneration within nodal culture systems (Table S1). Further research is required to deepen our understanding of the gene regulatory networks and identify key factors that can enhance callus and plant regeneration, particularly in recalcitrant

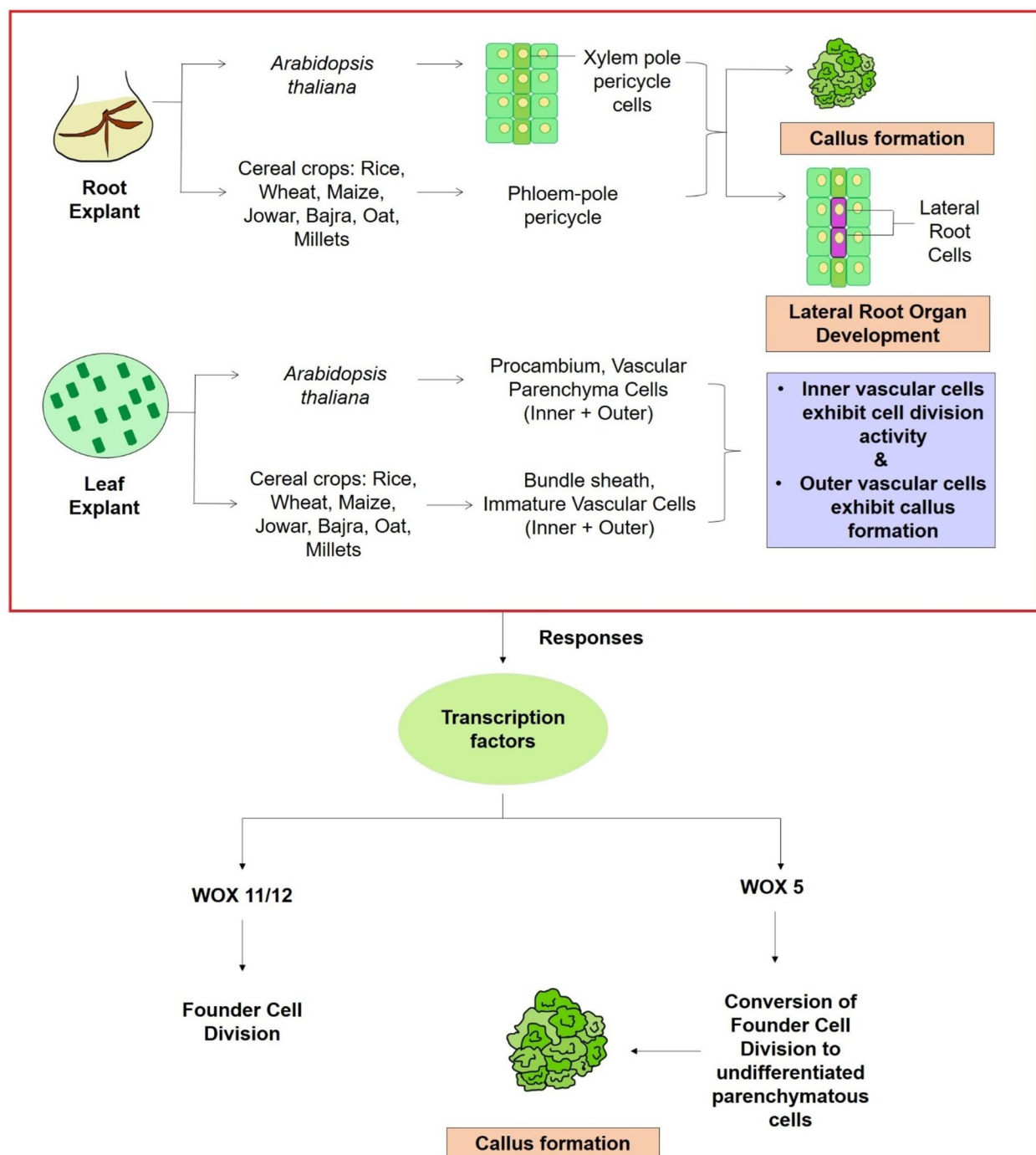


Fig. 4 Cellular mechanism in founder cells development, callus formation, and root growth. The figure illustrates the cellular and genetic responses of explants during founder cell formation, callus development, and root organogenesis. In root explants, the xylem-pole and phloem-pole pericycle cells play key roles in founder cell development and root organ formation. In leaf explants, structures such as procambium, vascular parenchyma, bundle sheath, and immature vascular cells are involved in cell division and callus formation. WOX 11 regulates founder cell division, while WOX 5 governs the transition from founder cells to callus formation. WOX5 or WOX11 and WOX12, WUSCHEL RELATED HOMEODOMAIN 5 or 11 and 12

crop species (Fig. 5). Given the conceptual and mechanistic overlap among various regeneration systems, it is scientifically plausible to hypothesize that these TFs

and signaling pathways also function in nodal culture-based regeneration, although direct functional validation remains an open research area. Recalcitrance in tissue

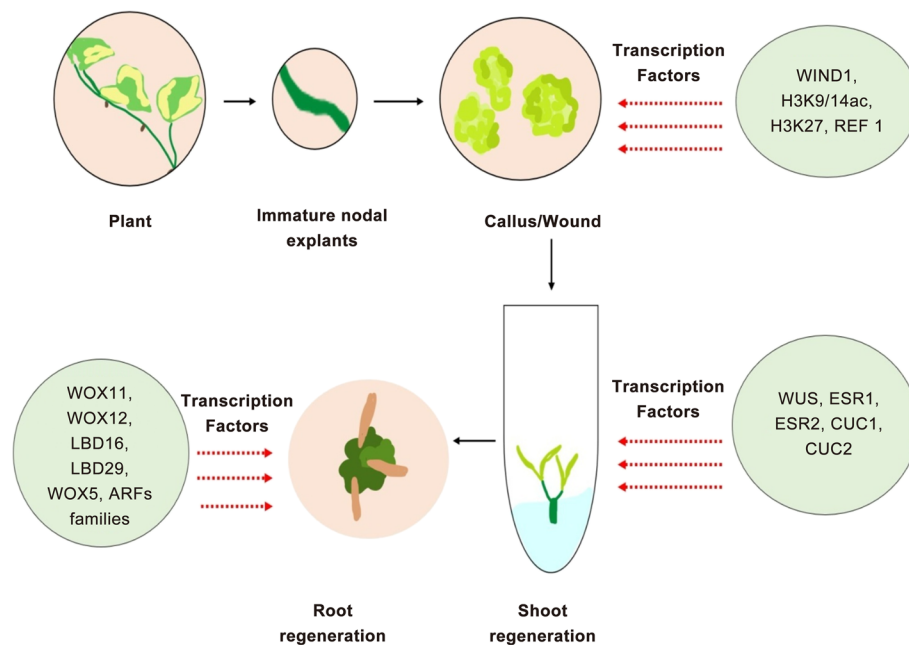


Fig. 5 Transcription factors in gene regulation for horticultural crop regeneration via nodal culture. This diagram illustrates key transcription factors (TFs) and gene networks involved in plant regeneration from nodal explants containing axillary meristems. In vitro, plant growth regulators (PGRs) such as cytokinins and auxins activate early-response TFs, triggering meristematic reprogramming. These TFs modulate genes critical for the maintenance of the shoot apical meristem (SAM) and axillary bud outgrowth. The figure emphasizes the coordinated transcriptional network driving shoot organogenesis from nodal tissues. WOX5 or WOX11 and WOX12, WUSCHEL RELATED HOMEODOMAIN 5 or 11 and 12; LBD16, LBD29, Lateral organ boundaries domain 16 or 29; ARF, auxin response factor; WIND1, Wound-Induced Dedifferentiation 1; Acetylation of lysine 9 on histone H3 (H3K9/14ac), H3K27, Methylation of histone H3 on lysine 27; WUS, Shoot meristem regulators WUSCHEL; ESR1, ESR2, Enhancer of Shoot Regeneration 1; CUC1, CUC2, Cup-shaped Cotyledon 1; REF1, regeneration factor 1

culture, especially during nodal explant regeneration, often arises from insufficient or delayed activation of key TFs involved in cell dedifferentiation, organogenesis, and meristem formation. Traditional methods, relying solely on external hormone supplementation (auxin, cytokinin), may fail to activate these transcriptional programs, particularly in species with inherently low regeneration capacity. A promising strategy to overcome this limitation is the use of CRISPR-based gene activation systems (CRISPRa), which can directly induce the expression of regeneration-associated transcription factors at the genomic level.

Integration of nodal culture with speed breeding techniques in recalcitrant horticultural crops

Speed breeding, an advanced technique, overcomes the limitations of traditional breeding by utilizing controlled environments to accelerate plant growth, particularly the transition from vegetative to reproductive stages. Concurrently, nodal culture enables rapid, clonal propagation while maintaining genotype integrity and producing disease-free crops, even in species with poor seed germination or rooting ability. The integration of these two strategies offers a promising

solution for recalcitrant crops, enhancing propagation speed, genetic improvement, and uniformity while overcoming challenges like low rooting capacity, contamination risks, and genotype specificity. When combined with speed breeding, nodal culture supports rapid turnover of growth cycles by optimizing factors such as photoperiod, light intensity, and CO₂ enrichment, all vital for accelerating both vegetative and reproductive growth (Potts et al. 2023). Immature nodal segments promote uniform regeneration responses, reducing variability and increasing experimental reproducibility. Moreover, the controlled conditions inherent to nodal culture reduce microbial contamination and physiological stress, supporting continuous propagation (Babu et al. 2022). By integrating these techniques, researchers can swiftly progress generations of recalcitrant crops, enabling faster selection of desirable traits and significantly reducing the time needed to achieve genetic improvements (Ben-nur et al. 2025). For example, in vitro cultured nodal segments generate healthy plantlets, which are then transitioned to speed breeding chambers for accelerated growth and flowering. This system is particularly beneficial for complex-genome crops like sugarcane,

where conventional breeding cycles are lengthy. Nodal culture provides uniform starting material, and speed breeding accelerates generation turnover, allowing researchers to complete multiple breeding cycles in a condensed timeframe. Thus, nodal culture complements speed breeding methodologies, advancing the rapid propagation and genetic enhancement of recalcitrant crops (Lone et al. 2020).

In this method, sterilized immature nodal segments from recalcitrant crops are cultured in specialized media to induce organized regeneration under controlled conditions. The regenerated populations are analyzed with advanced molecular techniques such as gene-specific primers, polymerase chain reaction (PCR), and autoradiography to distinguish between non-chimeric (fully transformed) and chimeric (partially transformed) individuals (Ahmad 2023). Non-chimeric plant lines are further advanced using speed breeding protocols within controlled environments to develop improved varieties and seed production. This approach accelerates the development of crops with enhanced traits, including improved resistance to biotic and abiotic stresses, biofortification, and better adaptation to climate-smart agricultural practices. Integrating non-chimeric regenerated populations into speed breeding systems ensures the production of healthy, high-performing crops under optimized conditions (Fig. 6) (Oyebamiji et al. 2024).

Integration of nodal culture with CRISPR/Cas-based gene editing systems

Traditional genetic modification methods often rely on tissue culture-based transformation, which proves inefficient for recalcitrant crops like sugarcane and certain tree species. The CRISPR/Cas system offers a precise alternative, enabling targeted gene editing without the need for labor-intensive tissue culture protocols, allowing for direct manipulation of genes of interest (Altaf et al. 2024). In crops where traditional tissue culture presents challenges, nodal culture serves as an effective platform for CRISPR/Cas-mediated genetic modifications, offering a viable solution for efficient regeneration of transgenic crops, particularly in recalcitrant species. This technique is especially advantageous for polyploid crops, such as sugarcane, where multiple genomes complicate conventional breeding approaches (Zhang and Yang 2024). CRISPR/Cas can precisely target specific haplotypes or alleles, facilitating more accurate genetic modifications. Moreover, CRISPR/Cas accelerates breeding by enabling targeted mutagenesis of genes associated with drought tolerance, disease resistance, and yield enhancement, thereby bypassing the extensive backcrossing and breeding cycles characteristic of traditional breeding (Rai

et al. 2023). Its high precision reduces off-target effects, ensuring that mutations are confined to the desired loci, a critical advantage for non-model or recalcitrant crops. Furthermore, CRISPR/Cas-based editing ensures biosafety by controlling gene flow and pollen transfer. Integrating CRISPR/Cas with nodal culture reduces the time, cost, and complexity of genetic modification in recalcitrant crops, unlocking new potential for accelerated crop improvement (Mishra and Pandey 2024). Additionally, regeneration via nodal culture can support the development of crops with enhanced resilience to both biotic and abiotic stresses, as well as improved nutritional qualities (Monthony 2021).

In this approach, sterilized immature nodal segments from recalcitrant horticultural crops are transformed using the CRISPR/Cas system. These transformed explants are cultured on basal media, supplemented with optimized concentrations of auxins, cytokinins, or organic additives, to induce direct or indirect plant regeneration under controlled environmental conditions (Bennur et al. 2025). Regenerated crops are evaluated using gene-specific primers, PCR, and autoradiography to differentiate between non-chimeric (fully transformed) and chimeric (partially transformed) individuals. Non-chimeric horticultural crops are then used to develop population lines with enhanced traits, such as improved stress tolerance, biofortification, and better seed germplasm quality (Fig. 7) (Chatterjee et al. 2025). Successful regeneration of gene-edited crops through nodal culture and the CRISPR/Cas system has been demonstrated in several recalcitrant species, including citrus, mini-citrus, and sugarcane (Kelly et al. 2025; Chatterjee et al. 2025) established an *in planta* genome-editing strategy for soil-grown citrus seedlings, utilizing *Agrobacterium tumefaciens* to transiently deliver three key functional modules: (i) a genome editing construct, (ii) a shoot regeneration module, and (iii) an enhanced T-DNA delivery system. The shoot regeneration module consists of a cassette encoding developmental regulators (DRs), specifically WUS (*ZmWUS2*) and STM (*AtSTM*), which promote *de novo* shoot organogenesis, enabling the recovery of genome-edited citrus crops directly from treated tissues. However, this combined approach has yet to be broadly applied to other recalcitrant crops or a wider range of agricultural and horticultural species. Expanding these methodologies could unlock new potential for efficient, non-chimeric regeneration in various economically significant species. Future research should focus on integrating nodal culture with CRISPR/Cas systems to enhance genetic transformation and regeneration efficiency in diverse recalcitrant agricultural and horticultural crops (Table 2). Incorporating speed breeding techniques alongside CRISPR/Cas in controlled environments could

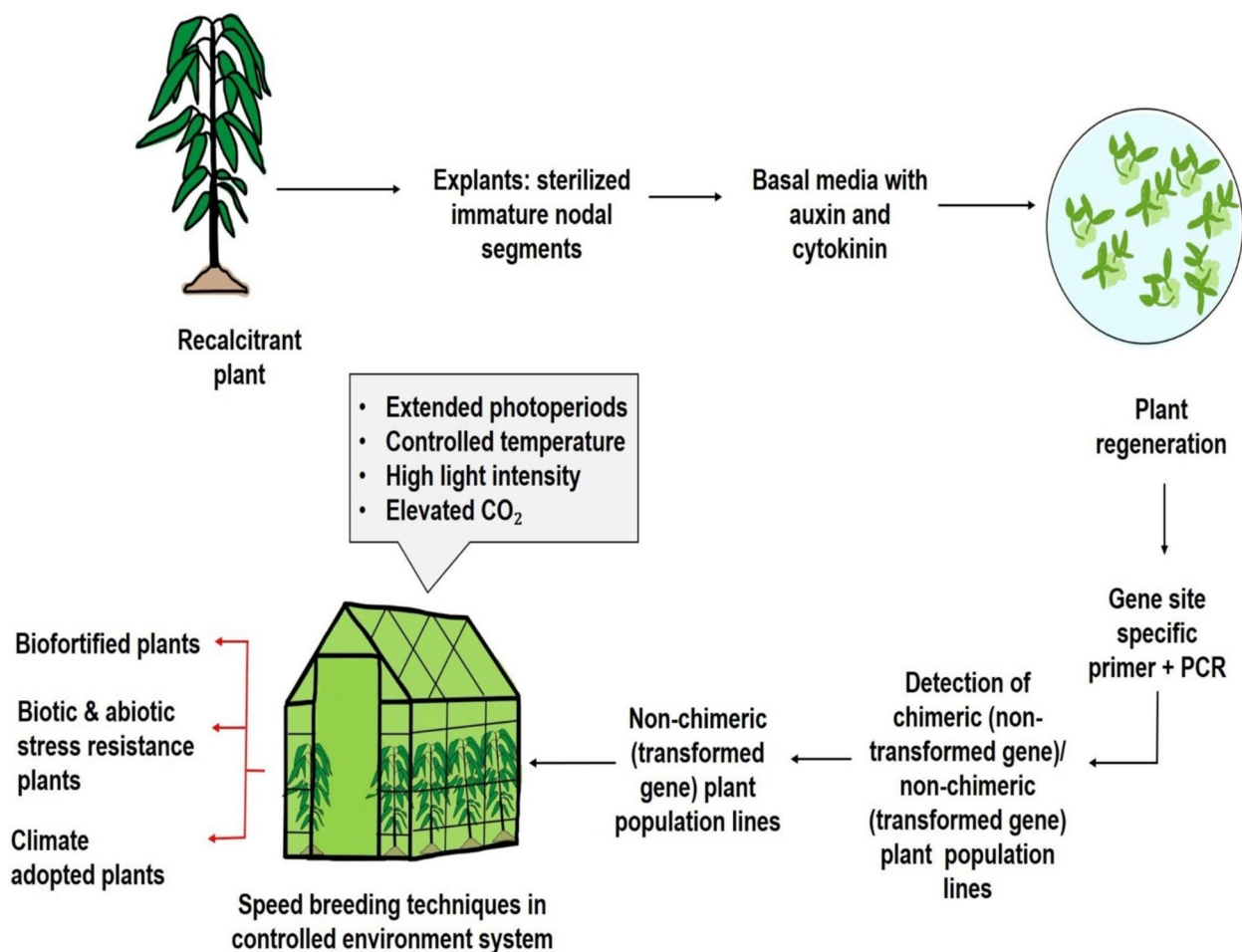


Fig. 6 Integrated approach for enhancing regeneration in recalcitrant horticultural crops using nodal culture and speed breeding. A streamlined strategy combining nodal culture with speed breeding to enhance regeneration and genetic transformation efficiency in recalcitrant plant species. Nodal explants from elite genotypes are cultured on optimized media under controlled conditions to induce direct or indirect organogenesis. Regenerated plantlets are then advanced through speed breeding protocols—extended photoperiods, controlled temperature, high light intensity, and elevated CO₂—to accelerate growth and generation turnover

further accelerate the development of improved crop varieties, contributing to global food security and climate change mitigation. Moreover, exploring soilless nodal culture techniques in controlled environments holds promise for advancing sustainable regenerative methods in agriculture (Monthony 2021).

Challenges in the nodal culture of recalcitrant horticultural crops

Nodal culture in recalcitrant crops poses several challenges that hinder efficient *in vitro* propagation and genetic enhancement. These crops often exhibit poor shoot and root induction due to intrinsic factors such as limited axillary bud activation and reduced regenerative potential. Success is further complicated by genotype dependence, the necessity for precise hormonal balance, and difficulties in adapting plant tissues to *in vitro*

conditions. A significant challenge is somaclonal variation, which can lead to undesirable genetic alterations during tissue culture, affecting the stability and uniformity of regenerated plants. Explant browning on solid media, often caused by oxidative stress, reduces cellular viability and hampers regeneration. Strategies to mitigate this include the incorporation of antioxidants like ascorbic acid or citric acid, subculturing, and maintaining low-light conditions. The adaptation and acclimatization phases are also sensitive to environmental fluctuations, which can negatively impact the survival of regenerated crops (Abdalla et al. 2022). Furthermore, the scarcity of key growth regulators such as auxins and cytokinins often necessitates co-culturing, allowing explants to share nutrients and signaling molecules, thereby enhancing both shoot and root regeneration. Without optimal media composition and growth regulator formulations,

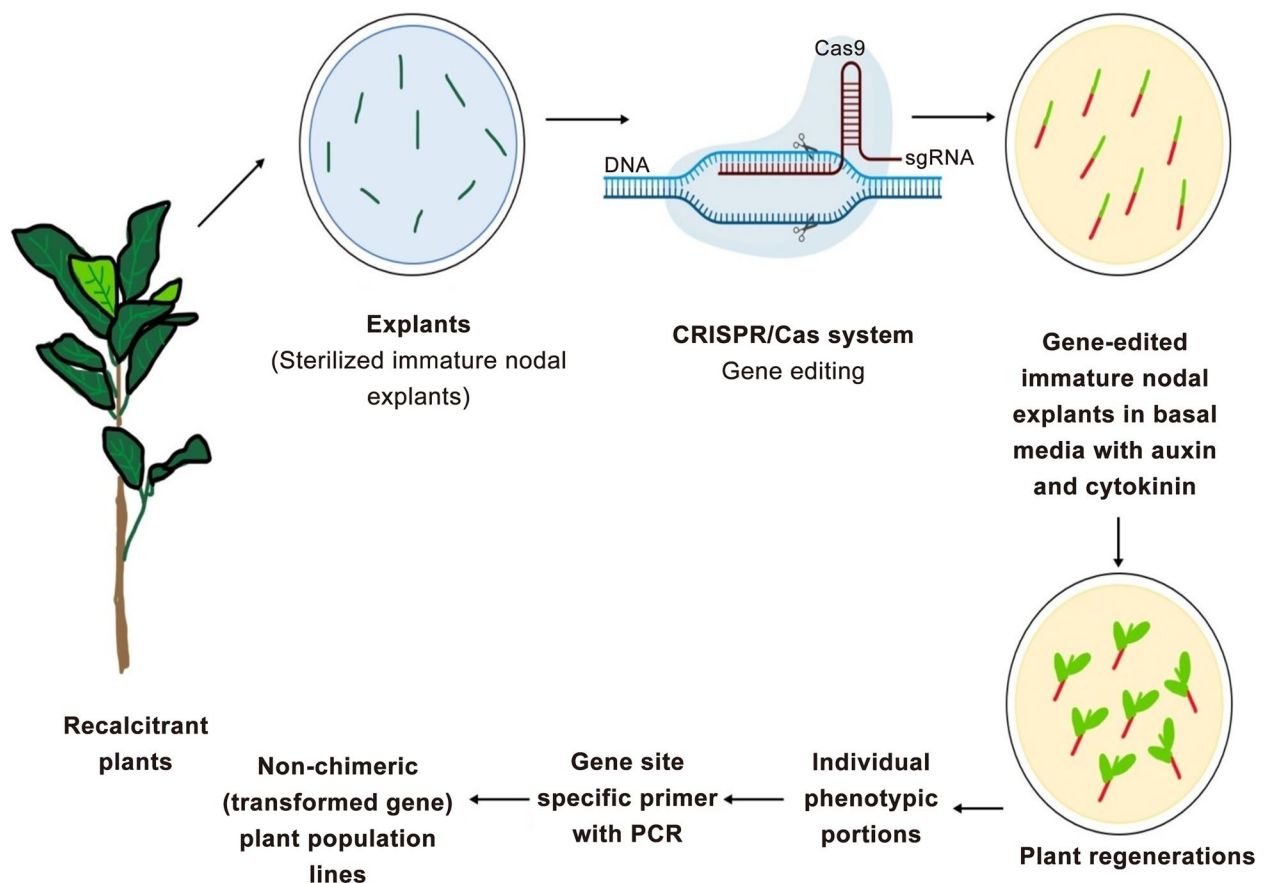


Fig. 7 Applications of the CRISPR/Cas system and nodal culture in recalcitrant horticultural crops. The schematic diagram illustrates a strategy utilizing nodal explants—commonly resistant to traditional transformation methods—as the foundation for CRISPR/Cas-driven genome editing. Nodal culture facilitates efficient shoot regeneration, with gene-editing constructs introduced via *Agrobacterium*, biolistics, or Polyethylene glycol (PEG). Edited nodes regenerate into whole plants, which are subsequently screened for successful edits through molecular and phenotypic analyses. This approach provides a viable solution for trait enhancement in crops with limited callus-based regeneration, such as woody perennials, legumes, and certain monocots

regeneration efficiency declines significantly (Coggins et al. 2014). The physiological age of explants also affects success rates, with older tissues generally exhibiting lower regenerative capacity (Lardon and Geelen 2020). Additionally, genetic variability in regenerated crops may alter critical traits, complicating the maintenance of desired phenotypes. Technical challenges, such as sterilization difficulties, contamination, and media optimization, further exacerbate the issues with nodal culture. Bacterial and fungal contamination, particularly from endophytes, remain persistent problems (Nair 2014). These microorganisms, often residing within plant tissues at the time of collection, are resistant to surface sterilization and can persist through multiple subcultures. Endophytic bacteria, commonly found in cell junctions or intercellular spaces of cortical parenchyma, are especially difficult to eliminate and can compromise long-term culture viability (Ahsan et al. 2025). Formulating an appropriate

culture medium is another critical challenge, as recalcitrant species have diverse nutritional requirements, demanding careful optimization of growth regulator types and concentrations (Gunson and Spencer-Phillips 1994). The slow growth rates and challenges in establishing stable cultures further contribute to the time-consuming nature of nodal culture in these species (Eziashi et al. 2014). Together, these biological and technical barriers emphasize the need for extensive protocol optimization and species-specific adjustments to enhance the success of nodal culture in recalcitrant crops.

Conclusions

Nodal culture, an advanced plant tissue culture technique, has demonstrated considerable success in regenerating various plant species under controlled conditions. However, despite its proven efficacy in horticultural species such as *Ixora* species, *Ixora fulgens*, *Citrus limon*

Table 2 Tissue cultured Plant regenerations with the interventions of Nodal Culture and CRISPR/Cas system

S.No	Crops	Explants	Methods	Targets	Results	References
	Mini-Citrus (<i>Fortunella hindsii</i> Swingle)	Nodal Segments-Axillary Buds	CRISPR/Cas9 + SgRNA + <i>Agrobacterium</i> mediated	Dwarf Plant Height, Early Flowering, Long Juvenile, Apomixis	96.9% TO-T1 Regeneration achieved 15 months generation time	Zhu et al. (2019)
	Citrus	Nodal Segment's	CRISPR/Cas9 + SgRNA + <i>Agrobacterium</i> mediated	Transgenic Population Line Generations	Advancement of transgenic population lines	Jia et al. (2019)
	Hong Kong kumquat (<i>Citrus hindsii</i> Champ. ex Benth.) Govaerts	Internodes	CRISPR/Cas9 + SgRNA + <i>Agrobacterium</i> mediated + qRT-PCR	Dwarf Plant Height, Pigment Enrichments, Short Internode	BBX22 and HY5 Controlled Internode Elongation, Pigments Enhancements, Dwarf Plant Height	Fu et al. (2024); Wang et al. (2024)
	Sweet orange- Carrizo citrange (<i>Citrus sinensis</i> L Osbeck x <i>Poncirus trifoliata</i> L. Raf.)	Internodal Segments	CRISPR/Cas9 + SgRNA + <i>Agrobacterium</i> mediated	Lycopene or Anthocyanin Enrichments	86% Nodal derived plantlets enriched with Lycopene and Anthocyanin	Salonia et al. (2022)
	Sugarcane (<i>Saccharum officinarum</i> L.)	Regenerated Nodal Segments	CRISPR/Cas9 + SgRNA + <i>Agrobacterium</i> mediated	Lignocelluloses Reductions	9.74–51.46% Lignin contents mitigated in the regenerated population lines	Laksana et al. (2024)

L., *Shorea robusta*, *Cucumis melo* L., *Avicennia marina*, *Persea americana*, *Coffea arabica*, and *Theobroma cacao* L., its integration with gene-editing platforms like the CRISPR/Cas system remains limited, especially in recalcitrant agricultural and horticultural crops. This represents a significant opportunity for innovation, as the synergistic combination of nodal culture and genome editing could dramatically improve regeneration efficiency and transformation success in challenging species. There is an urgent need to explore the regeneration potential of species with low in vitro responsiveness, refine genetic improvements in elite germplasm, and conserve both recalcitrant and climate-sensitive genotypes. These objectives are vital for addressing global food security, climate resilience, and sustainable agricultural development. Moreover, understanding the molecular and epigenetic barriers that limit regeneration competency in nodal tissues across diverse species is critical. There is also a gap in knowledge regarding the role of key transcription factors and responsive genes in mediating the complex transition from dedifferentiation to redifferentiation during direct and indirect regeneration pathways. Another key area of uncertainty is the precise role of ROS, which, in addition to functioning as stress signals, may influence hormonal crosstalk and cellular fate decisions through intricate signaling networks. Addressing these questions through integrative, interdisciplinary research is crucial for advancing nodal culture-based regeneration and its coupling with modern gene-editing technologies. One promising avenue is the application of synthetic biology to engineer customizable regulatory circuits that control transcriptional activation, hormone signaling, and ROS responses. The incorporation of synthetic promoters, inducible gene switches, and orthogonal transcription factor systems into nodal explants could fine-tune regeneration pathways in a species-specific manner. Furthermore, integrating nodal culture with speed breeding platforms and multi-omics analyses under controlled, soilless systems would accelerate trait development and enable functional validation of edited lines in horticultural crops. The convergence of nodal culture, CRISPR/Cas technology, and synthetic biology offers a transformative approach for improving recalcitrant species. This integrated platform not only enhances genetic advancements but also supports global efforts towards sustainable agriculture, food security, and climate resilience.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1007/s44281-025-00080-9>.

Supplementary Material 1.

Acknowledgements

Not applicable.

Authors' contributions

A.S., conceptualizations, draft manuscript preparations, compilation. H.P., V.M., conceptualizations, evaluation, directions, proofreading. V.M., figure making. B.C., M.S., evaluation, proofreading. R.K., P.H., V.S.D., A.K.M., A.V., N.T., evaluation, directions, proofreading.

Funding

There is no funding support and grant/award for this manuscript.

Data availability

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹ICAR-Indian Institute of Vegetable Research, Varanasi, Uttar Pradesh 221305, India. ²Division of Crop Improvement, ICAR-Indian Sugarcane Research Institute, Lucknow, Uttar Pradesh 226002, India. ³Faculty of Agricultural Sciences, Arunachal University of Studies, Namsai, Arunachal Pradesh 792103, India. ⁴KL College of Agriculture, KL Deemed To Be University, Vijayawada, Andhra Pradesh 522302, India. ⁵Division of Plant Physiology & Biochemistry, ICAR-Indian Sugarcane Research Institute, Lucknow, Uttar Pradesh 226002, India. ⁶College of Agriculture, Central Agricultural University, Imphal, Manipur 795004, India. ⁷School of Agriculture, Gandhi Institute of Engineering and Technology, Gunupur, Orissa 765022, India. ⁸College of Post-Harvest Technology and Food Processing, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh 250110, India. ⁹Department of Biotechnology, Vinayak Vidyapeeth College, Modipuram, Meerut 250110, India.

Received: 28 January 2025 Revised: 3 July 2025 Accepted: 4 July 2025

Published online: 10 October 2025

References

- Abdalla N, El-Ramady H, Seliem MK, El-Mahrouk ME, Taha N, Bayoumi Y, et al. An academic and technical overview on plant micropropagation challenges. *Horticulturae*. 2022;8:677. <https://doi.org/10.3390/horticulturae8080677>.
- Ahmad M. Plant breeding advancements with "CRISPR-Cas" genome editing technologies will assist future food security. *Front Plant Sci*. 2023;14:1133036. <https://doi.org/10.3389/fpls.2023.1133036>.
- Ahsan SM, Injamum-Ul-Hoque M, Das AK, Shaffique S, Hasan M, Kang SM, et al. Tuning up *in vitro* growth and development of *cannabis sativa*: recent advances in micropropagational approach. *Appl Biosci*. 2025;4(1): 12. <https://doi.org/10.3390/applbiosci4010012>.
- Akhatar J, Kaur H, Kumar H. Conventional plant breeding to modern biotechnological approaches in crop improvement. In: Kamaluddin, Kiran U, Abidin MZ, editors. *Technologies in: plant biotechnology and breeding of field crops*. Singapore: Springer Nature; 2022. p. 1–21. https://doi.org/10.1007/978-981-16-5767-2_1.
- Altaf MT, Liaqat W, Ali A, Jamil A, Bedir M, Nadeem MA, et al. Conventional and biotechnological approaches for the improvement of industrial crops. In: Kumar N, editor. *Industrial Crop Plants*. Singapore: Springer; 2024. p. 1–48. https://doi.org/10.1007/978-981-97-1003-4_1.
- Amin MN, Ahmad S, Sultana S, Alam MR, Azad MAK. In vitro rapid clonal propagation of an ornamental plant-*Ixora fulgens* Roxb. *J Biol Sci*. 2002;2(7):485. <https://doi.org/10.3923/jbs.2002.485.488>.

- Antwi-Wiredu A, Amiteye S, Diawuoh RG, Asumeng AK, Klu GY. In vitro propagation of rubber tree (*Hevea brasiliensis*) using shoot-tip and nodal cutting explants. *Int J Adv Struct Eng*. 2018;4(6):38–50. <https://doi.org/10.31695/IJASRE.2018.32743>.
- AV MM, Karishma NA. Development of micropropagation protocol for jackfruit (*Artocarpus heterophyllus* Lam.) KJ 182. *J Trop Agric*. 2024;62(1):10–13.
- Azam FMS, Rahmatullah M. Tissue culture of *Artocarpus heterophyllus* L., an underutilized fruit of Bangladesh. *Acta Hortic*. 2009;269–76.
- Babu GA, Mosa Christas K, Kowsalya E, Ramesh M, Sohn SI, Pandian S. Improved sterilization techniques for successful invitro micropropagation. In: Gupta S, Chaturvedi P, editors. *Commercial Scale Tissue Culture for Horticulture and Plantation Crops*. Singapore: Springer Nature; 2022. p. 1–21. https://doi.org/10.1007/978-981-19-0055-6_1.
- Barbedo CJ, Centeno DDC, Ribeiro RDCLF. Do recalcitrant seeds really exist? *Hoehnea*. 2013;40(4):583–93. <https://doi.org/10.1590/S2236-89062013000400001>.
- Belanger JG, Copley TR, Hoyos-Villegas V, Charron JB, O'Donoghue L. A comprehensive review of in planta stable transformation strategies. *Plant Methods*. 2024;20(1):79. <https://doi.org/10.1186/s13007-024-01200-8>.
- Bennur PL, O'Brien M, Fernando SC, Doblin MS. Improving transformation and regeneration efficiency in medicinal crops: Insights from other recalcitrant species. *J Exp Bot*. 2025;76(1):52–75. <https://doi.org/10.1093/jxb/erae189>.
- Berjak P, Pammenter NW. Physiology of desiccation-sensitive (Recalcitrant) seeds and the implications for cryopreservation. *Int J Plant Sci*. 2004;175:305–45. <https://doi.org/10.1086/673302>.
- Bhattacharyya R, Ghosh BN, Mishra PK, Mandal B, Rao CS, Sarkar D, et al. Soil degradation in India: challenges and potential solutions. *Sustainability*. 2015;7(4):3528–70. <https://doi.org/10.3390/su7043528>.
- Bidabadi SS, Jain SM. Cellular, molecular, and physiological aspects of invitro plant regeneration. *Plants*. 2020;9(6):702. <https://doi.org/10.3390/crops9060702>.
- Boboc Oros P, Cantor M, Cordea MI, Cătană C. Plant regeneration protocol for recalcitrant passion flower (*Passiflora quadrangularis* L.). *Horticulturae*. 2022;8(4): 337. <https://doi.org/10.3390/horticulturae8040337>.
- Cardoso JC, deOliveira MEB, deCardoso FC. Advances and challenges on the in vitro production of secondary metabolites from medicinal plants. *Hortic Bras*. 2019;37(2):124–32. <https://doi.org/10.1590/S0102-053620190201>.
- Chang W, Guo Y, Zhang H, Liu X, Guo L. Same actor in different stages: genes in shoot apical meristem maintenance and floral meristem determinacy in *Arabidopsis*. *Front Ecol Evol*. 2020;8:1–12. <https://doi.org/10.3389/fevo.2020.00089>.
- Chatfield SP, Capron R, Severino A, Penttilä PA, Alfred S, Nahal H, et al. Incipient stem cell niche conversion in tissue culture: using a systems approach to probe early events in wuschel-dependent conversion of lateral root primordia into shoot meristems. *Plant J*. 2013;73(5):798–813. <https://doi.org/10.1111/tpj.12085>.
- Chatterjee A, Purkaystha S, Bhattacharyya S, Sen P. Genetic engineering and genome editing for enhancing nutritional quality. In: Mathur P, Gupta A, editors. *Recent trends and applications of leguminous microgreens as Functional Foods*. Switzerland: Springer 2025;389–419. <https://doi.org/10.1007/978-3-031-75678-8>.
- Che P, Lall S, Nettelton D, Howell SH. Gene expression programs during shoot, root, and callus development in *Arabidopsis* tissue culture. *Plant Physiol*. 2006;141(2):620–37. <https://doi.org/10.1104/pp.106.081240>.
- Coggins CW Jr, Lovatt CJ. Plant growth regulators. In: Grafton-Cardwell B, editor. *Citrus production manual*. Oakland: University of California Agriculture and Natural Resources; 2014. p. 215.
- da Silva TL, Balzon TA, Scherwinski-Pereira JE. A rapid in vitro protocol for propagation of *Piper aduncum* and *Piper hispidinervum*, two species from Amazon region with multipurpose uses. *Afr J Biotechnol*. 2012;11(89):15539–46. <https://doi.org/10.5897/AJB12.1888>.
- Elhiti M, Stasolla C. ROS signalling in plant embryogenesis. In: Gupta KJ, Igamberdiev AU, editors. *Reactive oxygen and nitrogen species signaling and communication in crops*. Switzerland: Springer; 2014. p. 197–214. <https://doi.org/10.1007/978-3-319-10079-1>.
- Eziashi EI, Asemota O, Okwuagwu CO, Eke CR, Chidi NI, Oruade-Dimaro EA, et al. Screening sterilizing agents and antibiotics for the elimination of bacterial contaminants from oil palm explants for plant tissue culture. *Eur J Exp Biol*. 2014;4(4):111–5.
- Faizan M, Hayat S, Ahmed SM. *Reactive oxygen species: prospects in plant metabolism*. Singapore: Springer Nature; 2023. <https://doi.org/10.1007/978-981-19-9794-5>.
- Fambrini M, Usai G, Pugliesi C. Induction of somatic embryogenesis in crops: different players and focus on wuschel and wus-related homeobox (wox) transcription factors. *Int J Mol Sci*. 2022;23(24):15950. <https://doi.org/10.3390/ijms232415950>.
- Fan Y, Tang Z, Wei J, Yu X, Guo H, Li T, et al. Dynamic transcriptome analysis reveals complex regulatory pathway underlying induction and dose effect by different exogenous auxin IAA and 2, 4-d during invitro embryogenic redifferentiation in cotton. *Front Plant Sci*. 2022;13: 931105. <https://doi.org/10.3389/fpls.2022.931105>.
- Fernandez A, Leon-Lobos P, Contreras S, Ovalle JF, Serphen, Walt KVD, et al. The potential impacts of climate change on ex situ conservation options for recalcitrant-seeded species. *Front Glob Change*. 2023;6:1110431. <https://doi.org/10.3389/fgc.2023.1110431>.
- Fu J, Liao L, Jin J, Lu Z, Sun J, Song L, et al. A transcriptional cascade involving bbx22 and hy5 finely regulates both plant height and fruit pigmentation in citrus. *J Integr Plant Biol*. 2024;66(8):1752–68. <https://doi.org/10.1111/jipb.13719>.
- Gebremariam E. Invitro propagation of *Coffea arabica* cv. MCH2 using nodal explants. *J Nat Sci Res*. 2017;7(1):17–21.
- Goh T, Joi S, Mimura T, Fukaki H. The establishment of asymmetry in *Arabidopsis* lateral root founder cells is regulated by LBD16/ASL18 and related LBD/ASL proteins. *Development*. 2012;139(5):883–93. <https://doi.org/10.1242/dev.071928>.
- Gordon SP, Heisler MG, Reddy GV, Ohno C, Das P, Meyerowitz EM. Pattern formation during de novo assembly of the *Arabidopsis* shoot meristem. *Development*. 2007;134(19):3539–48. <https://doi.org/10.1242/dev.010298>.
- Goswami K, Sharma R, Singh PK, Singh G. Micropropagation of seedless lemon (*Citrus limon* L. cv. Kaghzi Kalan) and assessment of genetic fidelity of micropropagated plants using RAPD markers. *Physiol Mol Biol Plants*. 2013;19:137–45. <https://doi.org/10.1007/s12298-012-0148-0>.
- Guerriero G, Berni R, Muñoz-Sánchez JA, Apone F, Abdel-Salam EM, Qahtan AA, et al. Production of plant secondary metabolites: examples, tips and suggestions for biotechnologists. *Genes*. 2018;9(6): 309. <https://doi.org/10.3390/genes9060309>.
- Gunson HE, Spencer-Phillips PTN. Latent bacterial infections: epiphytes and endophytes as contaminants of micropropagated crops. In: Lumsden PJ, Nicholas JR, Davies WJ, editors. *Physiology, growth and development of crops in culture*. Dordrecht: Springer; 1994. p. 379–96. https://doi.org/10.1007/978-94-011-0790-7_43.
- Hill K, Schaller GE. Enhancing plant regeneration in tissue culture. *Plant Signal Behav*. 2013;8(10): e25709. <https://doi.org/10.4161/psb.25709>.
- Hiti-Bandaralage JC, Hayward A, Mitter N. Micropropagation of avocado (*Persea americana* Mill.). *Am J Plant Sci*. 2017;11:2898–921. <https://doi.org/10.4236/ajps.2017.811197>.
- Hoang XLT, Prerostova S, Thu NBA, Thao NP, Vankova R, Tran LSP. Histidine kinases: diverse functions in plant development and responses to environmental conditions. *Annu Rev Plant Biol*. 2021;72:297–323. <https://doi.org/10.1146/annurev-arplant-080720-093057>.
- Hu B, Zhang G, Liu W, Shi J, Wang H, Qi M, et al. Divergent regeneration-competent cells adopt a common mechanism for callus initiation in angiosperms. *Regeneration*. 2017;4(3):132–9. <https://doi.org/10.1002/reg.282>.
- Ikeuchi M, Ogawa Y, Iwase A, Sugimoto K. Plant regeneration: cellular origins and molecular mechanisms. *Development*. 2016;143(9):1442–51. <https://doi.org/10.1242/dev.134668>.
- Ikeuchi M, Favero DS, Sakamoto Y, Iwase A, Coleman D, Ryman B, et al. Molecular mechanisms of plant regeneration. *Annu Rev Plant Biol*. 2019;70:377–406. <https://doi.org/10.1146/annurev-arplant-050718-100434>.
- Isah T, Umar S, Mujib A, Sharma MP, Rajasekharan PE, Zafar N, et al. Secondary metabolism of pharmaceuticals in the plant *in vitro* cultures: strategies, approaches, and limitations to achieving higher yield. *Plant Cell, Tissue and Organ Culture (PCTOC)*. 2018;132:239–65. <https://doi.org/10.1007/s11240-017-1332-2>.
- Isda MN, Hasibuan M, Fatimah S. The formation of shoots in mangosteen (*Garcinia Mangostana* L.) nodules from Bengkalis, Riau in Murashige Skoog

- and woody plant medium. *J Phys Conf Ser*. 2019;1351:12–28. <https://doi.org/10.1088/1742-6596/1351/1/012028>.
- Iwase A, Mitsuda N, Koyama T, Hiratsu K, Kojima M, Arai T, et al. The Ap2/erf transcription factor Wind1 controls cell dedifferentiation in Arabidopsis. *Curr Biol*. 2011;21(6):508–14. <https://doi.org/10.1016/j.cub.2011.02.020>.
- Iwase A, Mita K, Nonaka S, Ikeuchi M, Koizuka C, Ohnuma M, et al. Wind1-based acquisition of regeneration competency in Arabidopsis and rapeseed. *J Plant Res*. 2015;128:389–97. <https://doi.org/10.1007/s10265-015-0714-y>.
- Jain S, Singh H, Rathod M, Meena R, Deshmukh RN, Mohapatra A, et al. Preserving for the future: the critical role of germplasm conservation in fruit crop resilience. *Int J Environ Clim Change*. 2023;13(11):4651–61. <https://doi.org/10.9734/ijec/2023/v13i113644>.
- Jia H, Zou X, Orbovic V, Wang N. Genome editing in citrus tree with CRISPR/Cas9. *Plant genome editing with CRISPR systems: Methods and Protocols*. 2019;235–241. <https://doi.org/10.1111/pbi.13132>.
- Jose S, Thomas TD. Abiotic stresses increase plant regeneration ability of rhizome explants of *Curcuma caesia* Roxb. *Plant Cell Tissue Organ Cult*. 2015;122:767–72. <https://doi.org/10.1007/s11240-015-0795-2>.
- Kareem A, Durgaprasad K, Sugimoto K, Du Y, Pulianmackal AJ, Trivedi ZB, et al. Plethora genes control regeneration by a two-step mechanism. *Curr Biol*. 2015;25(8):1017–30. <https://doi.org/10.1016/j.cub.2015.02.022>.
- Kelly G, Plesser E, Bdoach E, Arroyave M, Belausov E, Doron-Faigenboim A, et al. In plant genome editing in citrus facilitated by co-expression of CRISPR/Cas and developmental regulators. *Plant J*. 2025;122(2): e70155. <https://doi.org/10.1111/tpj.70155>.
- Kim DH, Gopal J, Sivanesan I. Nanomaterials in plant tissue culture: the disclosed and undisclosed. *RSC Adv*. 2017;7(58):36492–505. <https://doi.org/10.1039/C7RA07025J>.
- Kirch T, Simon R, Grünewald M, Werr W. The dornroschen/Enhancer of shootregeneration1 gene of arabidopsis acts in the control of meristem cell fate and lateral organ development. *Plant Cell*. 2003;15(3):694–705. <https://doi.org/10.1105/tpc.009480>.
- Kwiatkowska D. Flowering and apical meristem growth dynamics. *J Exp Bot*. 2008;59(2):187–201. <https://doi.org/10.1093/jxb/erm290>.
- Laksana C, Sophiphun O, Chanprame S. Lignin reduction in sugarcane by performing CRISPR/Cas9 site-direct mutation of *SoLIM* transcription factor. *Plant Sci*. 2024;340: 111987. <https://doi.org/10.1016/j.plantsci.2024.111987>.
- Lal R, Smith P, Jungkunst HF, Mitsch WJ, Lehmann J, Nair PR, et al. The carbon sequestration potential of terrestrial ecosystems. *J Soil Water Conserv*. 2018;73(6):145A–152A. <https://doi.org/10.2489/jswc.73.6.145A>.
- Lardon R, Geelen D. Natural variation in plant pluripotency and regeneration. *Plants*. 2020;9(10):1261. <https://doi.org/10.3390/plants9101261>.
- Lee JS. To overcome the limitations of fixed life patterns, crops can generate meristems throughout life. *J Plant Physiol*. 2023;291: 154097. <https://doi.org/10.1016/j.jplph.2023.154097>.
- Li J, Zhang Q, Wang Z, Liu Q, et al. The roles of epigenetic regulators in plant regeneration: exploring patterns amidst complex conditions. *Plant Physiol*. 2024;194:2022–38. <https://doi.org/10.1093/plphys/kiad042>.
- Liu J, Sheng L, Xu Y, Li J, Yang Z, Huang H, et al. WOX11 and 12 are involved in the first-step cell fate transition during de novo root organogenesis in Arabidopsis. *Plant Cell*. 2014;26(3):1081–93. <https://doi.org/10.1105/tpc.114.122887>.
- Lone SM, Hussain K, Malik A, Magray M, Hussain SM, Rashid M, et al. Plant propagation through tissue culture—a biotechnological intervention. *Int J Curr Microbiol App Sci*. 2020;9(7):2176–90. <https://doi.org/10.20546/ijcmas.2020.907.254>.
- Long Y, Yang Y, Pan G, Shen Y. New insights into tissue culture plant-regeneration mechanisms. *Front Plant Sci*. 2022;13: 926752. <https://doi.org/10.3389/fpls.2022.926752>.
- Luo Z, Xu X, Shao T, Zhang J, Xu W, Yao J, et al. ROS-induced autophagy regulates porcine trophectoderm cell apoptosis, proliferation, and differentiation. *Am J Physiol Cell Physiol*. 2019;316(2):C198–209. <https://doi.org/10.1152/ajpcell.00256.2018>.
- Mahmad N. Germination, regeneration and pigment detection in *Nelumbo nucifera* gaertn. (pink Asian lotus) [Doctoral dissertation]. Kuala Lumpur: Institute of Biological Sciences, University of Malaya; 2012. p. 1–404.
- Majumder A, Roychowdhury D, Ray S. Biotechnological approaches for ex situ conservation of medicinal crops. In: Jha S, Halder M, editors. *Medicinal Plants: biodiversity, biotechnology and conservation*. Singapore: Springer; 2023. p. 729–800. https://doi.org/10.1007/978-981-19-9936-9_26.
- Mazri MA, Koufan M, Abdelwahd R, Belkoura I. In vitro responses of some Mediterranean fruit crops to auxin, cytokinin and gibberellin treatments. In: Aftab T, editor. *Auxins, cytokinins and gibberellins signaling in crops*. Singapore: Springer; 2022. p. 91–123. https://doi.org/10.1007/978-3-031-05427-3_5.
- Mishra A, Pandey VP. CRISPR/Cas system: a revolutionary tool for crop improvement. *Biotechnol J*. 2024;19(2): 2300298. <https://doi.org/10.1002/biot.202300298>.
- Monthony AS, Bagheri S, Zheng Y, Jones AMP. Weeding out challenges in cannabis tissue culture and developing novel micropropagation and regeneration methods [Doctoral dissertation]. Guelph: University of Guelph; 2021. p. 1–143.
- Mosoh DA, Khandel AK, Verma SK, Vendrame WA. Multi-explant and multiplex applications of plant growth regulators: A critical analysis of direct organogenesis in *Gloriosa superba* (L.). *Tropical Plants*. 2024;3: e039. <https://doi.org/10.48130/tp-0024-0038>.
- Motte H, Vercauteren A, Depuydt S, Landschoot S, Geelen D, Werbrouck S, et al. Combining linkage and association mapping identifies receptor-like protein kinase1 as an essential Arabidopsis shoot regeneration gene. *Proc Natl Acad Sci U S A*. 2014;111(22):8305–10. <https://doi.org/10.1073/pnas.1404978111>.
- Nair DN, Padmavathy S. Impact of endophytic microorganisms on crops, environment and humans. *Sci World J*. 2014;2014(1): 250693. <https://doi.org/10.1155/2014/250693>.
- Ochatt SJ. Less frequently used growth regulators in plant tissue culture. In: Loyola-Vargas V, Ochoa-Alejo N, editors. *Plant cell culture protocols*. New York: Springer; 2024. p. 109–143. https://doi.org/10.1007/978-1-0716-3954-2_8.
- Oyebamiji YO, Adigun BA, Shamsudin NAA, Ikmal AM, Salisu MA, Malike FA, et al. Recent advancements in mitigating abiotic stresses in crops. *Horticulturae*. 2024;10(2):156. <https://doi.org/10.3390/horticulturae10020156>.
- Parvin S, Kausar M, Haque ME, Khalekuzzaman M, Sikdar B, Islam MA. In vitro propagation of muskmelon (*Cucumis melo* L.) from nodal segments, shoot tips and cotyledonary nodes. *Rajshahi Univ J Life Earth Agril Sci*. 2013;41:71–7. <https://doi.org/10.3329/rujles.v41i021627>.
- Pasternak TP, Steinmacher D. Plant growth regulation in cell and tissue culture in vitro. *Plants*. 2024;13(2): 327. <https://doi.org/10.3390/plants13020327>.
- Patel P, Sarswat SK, Modi A. Strategies to overcome explant recalcitrance under in vitro conditions. In: Rai AC, Kumar A, Modi A, Singh M, editors. *Advances in plant tissue culture*. Cambridge: Academic Press; 2022. p. 283–94. <https://doi.org/10.1016/B978-0-323-90795-8.00003-5>.
- Perera D, Barnes DJ, Baldwin BS, Reichert NA. Direct and indirect in vitro regeneration of *Miscanthus x giganteus* cultivar Freedom: effects of explant type and medium on regeneration efficiency. *In Vitro Cell Dev Biol Plant*. 2015;51:294–302. <https://doi.org/10.1007/s11627-015-9682-0>.
- Pérez-Tornero O, Tallón CI, Porras I. An efficient protocol for micropropagation of lemon (*Citrus limon*) from mature nodal segments. *Plant Cell Tissue Organ Cult*. 2010;100:263–71. <https://doi.org/10.1007/s11240-009-9643-6>.
- Potts J, Jangra S, Michael VN, Wu X. Speed breeding for crop improvement and food security. *Crops*. 2023;3(4):276–91. <https://doi.org/10.3390/crops3040025>.
- Pritchard HW, Tsan FY, Wen B, Jaganathan GK, Calvi G, Pence VC, et al. Regeneration in recalcitrant-seeded species and risks from climate change. In: Baskin CC, Baskin JM, editors. *Plant regeneration from seeds: a global warming perspective*. Cambridge: Academic Press; 2022. p. 259–73. <https://doi.org/10.1016/C2020-0-00735-8>.
- Pujalon S, Piola F, Bornette G. Abiotic stresses increase plant regeneration ability. *Evol Ecol*. 2008;22:493–506. <https://doi.org/10.1007/s10682-007-9177-5>.
- Pumisutapon P, Visser RGF, de Klerk GJ. Moderate abiotic stresses increase rhizome growth and outgrowth of axillary buds in *Alstroemeria* cultured in vitro. *Plant Cell Tissue Organ Cult*. 2012;110:395–400. <https://doi.org/10.1007/s11240-012-0160-7>.
- Rai GK, Khanday DM, Kumar P, Magotra I, Choudhary SM, Kosser R, et al. Enhancing crop resilience to drought stress through CRISPR-Cas9 genome editing. *Plants*. 2023;12(12):2306. <https://doi.org/10.3390/plant12122306>.

- Ramos LCDS, Almeida JASD. Effect of 6-BA on nodal explant bud sproutings of *Coffea arabica* cv. Mundo Novo. *Bragantia*. 2005;64(2):185–90. <https://doi.org/10.1590/S0006-87052005000200003>.
- Rasheed H, Shi L, Winarsih C, Jakada BH, Chai R, Huang H. Plant growth regulators: an overview of wox gene family. *Plants*. 2024;13(21):3108. <https://doi.org/10.3390/crops13213108>.
- Ravindran PN, Nirmal Babu K, Shiva KN. Botany and crop improvement of turmeric. In: Ravindran PN, Nirmal Babu K, Sivaraman K, editors. *Turmeric: the genus Curcuma*. London: CRC Press; 2007. p. 15–70. <https://doi.org/10.1201/9781420006322>.
- Ricroch A, Dervishi A, Choudry MW, Riaz R, Bakhsh A. A roadmap for plant genome editing. *Springer*, Singapore; 2024. p. 259–69. <https://library.oapen.org/handle/20.500.12657/86933>.
- Riou-Khamlich C, Huntley R, Jacquemard A, Murray JAH. Cytokinin activation of Arabidopsis cell division through a D-type cyclin. *Science*. 1999;283(5407):1541–4. <https://doi.org/10.1126/science.283.5407.1541>.
- Roy SK, Roy PK, Sinha P, Haque MS. Mass clonal propagation of *Artocarpus heterophyllus* through in vitro culture. In: Kubota C, Chun C, editors. *Transplant production in the 21st century*. Dordrecht: Springer; 2000. p. 219–25. https://doi.org/10.1007/978-94-015-9371-7_36.
- Rupp HM, Frank M, Werner T, Strnad M, Schmuelling T. Increased steady state mRNA levels of the *stm* and *knot1* homeobox genes in cytokinin overproducing Arabidopsis thaliana indicate a role for cytokinins in the shoot apical meristem. *Plant J*. 1999;18(5):557–63. <https://doi.org/10.1046/j.1365-3113.1999.00472.x>.
- Salonia F, Ciacciulli A, Pappalardo HD, Poles L, Pindo M, Larger S, et al. A dual sgRNA-directed CRISPR/Cas9 construct for editing the fruit-specific β -cyclase 2 gene in pigmented citrus fruits. *Front Plant Sci*. 2022;13:975917. <https://doi.org/10.3389/fpls.2022.975917>.
- Sehgal H, Joshi M. The journey and new breakthroughs of plant growth regulators in tissue culture. In: Rai AC, Kumar A, Modi A, Singh M, editors. *Advances in plant tissue culture*. Cambridge: Academic Press; 2022. p. 85–108. <https://doi.org/10.1016/B978-0-323-90795-8.00002-3>.
- Shields R, Robinson S, Anslow PA. Use of fungicides in plant tissue culture. *Plant Cell Rep*. 1984;3:33–6. <https://doi.org/10.1007/BF00270226>.
- Sholi N, Qasrawi H. In vitro regeneration of avocado (*Persea americana*) west indian rootstock cv. lula via tissue culture. *مجلة جامعة فلسطين التقنية للابحاث*. 21–11;(1)10;2022.
- Silveira SS, Cordeiro-Silva R, Degenhardt-Goldbach J, Quoirin M. Micropropagation of *Calophyllum brasiliense* (Cambess.) from nodal segments. *Braz J Biol*. 2016;76(3):656–63. <https://doi.org/10.1590/1519-6984.23714>.
- Singh M, Sonkusale S, Niratker CH, Shukla P. Micropropagation of *Shorea robusta*: an economically important woody plant. *J for Sci*. 2014;60(2):70–4. <https://doi.org/10.17221/80/2013-JFS>.
- Singh P, Singh BK, Singh SP, Padhi M. Micropropagation of Kinnow mandarin using nodal segments as explants. *J Pharmacogn Phytochem*. 2018;7(4):2224–6.
- Sivakumar P, Pavithra N, Ballolla T, Vineeta ME, Selvaraj KV, Logeswaran J. Effect of growth regulators in invitro propagation of *Ixora* spp.: an short review. *J Curr Opin Crop Sci*. 2020;1(1):31–4. <https://doi.org/10.62773/jcocs.v1i1.15>.
- Sujith SS. In vitro techniques for conservation of recalcitrant species [Master's Seminar]. Thrissur: Centre for plant biotechnology and molecular biology, College of Horticulture, Kerala Agricultural University; 2015. p. 1–35.
- To JP, Haberer G, Ferreira FJ, Deruère J, Mason MG, Schaller GE, et al. Type-A Arabidopsis response regulators are partially redundant negative regulators of cytokinin signaling. *Plant Cell*. 2014;16(3):658–71. <https://doi.org/10.1105/tpc.018978>.
- Traore A, Maximova SN, Gultinan MJ. Micropropagation of Theobroma cacao L. using somatic embryo-derived plants. *In Vitro Cellular & Developmental Biology-Plant*. 2003;39(3):332–7. <https://doi.org/10.1079/IVP2002409>.
- Wang S, Zhao S, Du M, Mo K, Pang S, Zou X. Development and application of gene editing in citrus: how and what to do. *Hortic Adv*. 2024;2: 31. <https://doi.org/10.1007/s44281-024-00053-4>.
- Yang Q, Yuan C, Cong T, Zhang Q. The secrets of meristems initiation: axillary meristem initiation and floral meristem initiation. *Plants*. 2023;12(9):1879. <https://doi.org/10.3390/plants12091879>.
- Zeng M, Hu B, Li J, Zhang G, Ruan Y, Huang H, et al. Stem cell lineage in body layer specialization and vascular patterning of rice root and leaf. *Sci Bull*. 2016;61(11):847–58. <https://doi.org/10.1007/s11434-015-0849-1>.
- Zhai N, Xu L. Pluripotency acquisition in the middle cell layer of callus is required for organ regeneration. *Nat Plants*. 2021;7:1453–60. <https://doi.org/10.1038/s41477-021-01015-8>.
- Zhang S, Lemaux PG. Molecular analysis of in vitro shoot organogenesis. *CRC Crit Rev Plant Sci*. 2004;23(4):325–35. <https://doi.org/10.1080/07352680490484569>.
- Zhang L, Yang X. Advances in sugarcane genomics: navigating through complex polyploid genomes. *Mol Plant Breed*. 2024;15(4):155–66. <https://doi.org/10.5376/mpb.2024.15.0016>.
- Zhu C, Zheng X, Huang Y, Ye J, Chen P, Zhang C, et al. Genome sequencing and CRISPR/Cas9 gene editing of an early flowering mini-citrus (*Fortunella hindsii*). *Plant Biotechnol J*. 2019;17(11):2199–210. <https://doi.org/10.1111/pbi.13132>.
- Zhumanova N, Akimbayeva N, Myrzakhmetova N, Dzhiembayev B, Ku A, Diyarova B, et al. A comprehensive review of new generation plant growth regulators. *ES Food & Agrofor*. 2024;17:1190. <https://doi.org/10.30919/esf1190>.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.