

Overcoming Challenges in Commercial-Scale Micropropagation of Sugarcane: Strategies and Solutions

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Abstract

Sugarcane (*Saccharum officinarum* L.) is a vital crop for global agriculture, contributing significantly to sugar production, bioenergy, and various industrial applications. Despite its economic importance, large-scale production of high-quality, disease-free sugarcane plants through conventional propagation methods remains a challenge due to low multiplication rates and susceptibility to pests and diseases. Micropropagation offers a powerful alternative for rapid clonal multiplication of elite varieties, ensuring uniformity and improved productivity. However, the commercialization of sugarcane micropropagation faces numerous constraints, including genotype-dependent responses, somaclonal variation, high production costs, and labor-intensive protocols. This review explores these constraints in detail and discusses innovative strategies to overcome them. Key advancements include the optimization of culture media, the strategic use of plant growth regulators, the adoption of bioreactor systems for large-scale production, and the implementation of cryopreservation for germplasm conservation. Additionally, cost-effective approaches such as automation, scaling up of bioreactor-based methods, and improved acclimatization protocols are highlighted as solutions to enhance efficiency and reduce expenses. By addressing these challenges, commercial-scale sugarcane micropropagation can revolutionize the industry, providing

sustainable and scalable solutions to meet the growing global demand for sugarcane and its derivatives.

Keywords : Genetic Engineering, Stress tolerance, In vitro culture, Mutagenesis, Transgenic plants

Introduction

Sugarcane (*Saccharum* spp. hybrids) stands as a globally vital crop, primarily cultivated for sucrose, bioethanol, and bioenergy production. Traditional propagation using stem cuttings (setts) suffers from severe limitations: slow multiplication rates (typically 1:5-10 per year), high susceptibility to systemic diseases (e.g., ratoon stunting disease - *Leifsonia xyli* subsp. *xyli*, leaf scald - *Xanthomonas albilineans*, smut - *Sporisorium scitamineum*), accumulation of pests, and significant transportation costs for bulky planting material. Micropropagation (tissue culture) offers a transformative solution, enabling the rapid production of large quantities of genetically uniform, disease-free, high-quality plantlets from elite clones. The theoretical benefits include exponential multiplication rates, elimination of systemic pathogens, enhanced field uniformity, accelerated release of new varieties, and year-round nursery production. However, scaling micropropagation from the laboratory bench to economically viable, consistent commercial production faces significant biological, technical, and economic hurdles. Overcoming these challenges is critical for realizing the full potential of this technology to revolutionize sugarcane cultivation (Snyman et al., 2011; Srivastava et al., 2016).

Challenge 1: Contamination Control at Scale

Contamination by bacteria, fungi, and yeasts is a primary bottleneck in commercial micropropagation. Sugarcane stems, the primary explant source, harbor diverse endogenous microbes (endophytes) residing deep within tissues, making surface sterilization ineffective. These contaminants often proliferate unseen initially, causing explant death, culture loss, and production delays during critical multiplication phases. Scaling exacerbates the problem due to the sheer volume of cultures handled. Key strategies involve source plant management (growing stock plants under protected

conditions with reduced pesticide use to minimize surface contaminants), exploit selection (using meristem tips which often have lower endophyte load compared to larger nodal segments), enhanced sterilization protocols (sequential treatments with fungicides, bactericides, and oxidizing agents like NaOCl, often combined with vacuum infiltration or prolonged antibiotic soaks), and culture indexing (regularly testing apparently clean cultures on rich bacteriological/fungal media to detect latent contaminants). The strategic use of broad-spectrum antibiotics and fungicides incorporated into initiation media (e.g., Cefotaxime, Kanamycin, Carbenicillin, Benomyl) is common, though concerns about phytotoxicity, development of resistant strains, and cost necessitate careful optimization and rotation. Rigorous aseptic technique automation (where feasible) and strict environmental control in culture rooms are paramount (Grisham et al., 2009; Taylor et al., 2010; Lal et al., 2015).

Challenge 2: Managing Phenolic Exudation and Browning

Upon excision and placement in culture, sugarcane tissues rapidly exude phenolic compounds (e.g., chlorogenic acid, caffeic acid derivatives) which oxidize, turning the medium brown and releasing phytotoxic quinones. This browning severely inhibits explant establishment, shoot initiation, and subsequent growth, leading to high mortality. The problem is genotype-dependent but prevalent. Mitigation strategies focus on antioxidant application: adding compounds like activated charcoal (AC: 0.1-3.0%), polyvinylpyrrolidone (PVP: 100-500 mg/L), ascorbic acid (50-200 mg/L), citric acid (50-150 mg/L), or L-cysteine (20-100 mg/L) to the initiation medium to adsorb or reduce phenolics. Frequent subculture during the initial establishment phase (every 3-7 days) physically removes explants from the toxic medium. Culture condition optimization such as incubation in darkness or low light for the first week reduces oxidation rates. Using liquid overlay systems (a thin layer of liquid medium over semi-solid) or temporary immersion bioreactors (TIBs) during initiation can also dilute phenolics more effectively than static semi-solid media (Lorenzo et al., 2001; Ali et al., 2007; Khan et al., 2017).

Challenge 3: Minimizing Somaclonal Variation

While micropropagation aims for clonal fidelity, sugarcane's complex polyploid and aneuploid genome makes it particularly prone to somaclonal variation – heritable genetic and epigenetic changes arising during tissue culture. This manifests as off-types in the field, exhibiting variations in yield, sucrose content, disease susceptibility, stalk morphology, and ratooning ability, undermining the value of clonal uniformity. Strategies focus on propagation pathway selection: prioritizing axillary bud proliferation from shoot tips or nodal buds over callus-mediated organogenesis or somatic embryogenesis, as the latter significantly increases variation risk. Limiting subculture cycles (typically to 8-12 cycles maximum) prevents the accumulation of genetic errors. Careful clone selection: some genotypes are inherently more stable than others; screening parental clones for stability is crucial. Implementing rigorous quality control protocols involving molecular markers (SSRs, AFLPs, RAPDs, SNPs) for genetic fingerprinting at various stages (mother plant, culture bank, hardened plants) allows early detection of variants. Field roguing remains essential, where micropropagated plant crops (MPC) are meticulously inspected during early growth stages to remove phenotypic off-types before they contribute to further propagation (Krishnareddy, 2013; Jaligot et al., 2014; Ramdoyal et al., 2019).

Challenge 4: Controlling Hyperhydricity (Vitrification)

Hyperhydricity results in shoots becoming translucent, swollen, brittle, and waterlogged due to disrupted lignification and abnormal stomatal function. These shoots exhibit poor growth, root poorly, and have very low survival rates during acclimatization. Factors promoting hyperhydricity include high humidity inside vessels, high cytokinin concentrations (especially BAP), low light intensity, ammonium ion accumulation, and poor gas exchange. Solutions involve optimizing gelling agents: increasing agar concentration (0.8-1.0%) or using superior gelling agents like Gelrite®/Phytigel™ (0.2-0.3%) to reduce water availability. Vessel ventilation: using lids with gas-permeable membranes or leaving closures slightly loose enhances gas exchange and

reduces humidity. Growth regulator management: reducing cytokinin levels (BAP) and duration of exposure, often substituting with lower concentrations of kinetin or metatopolin, and incorporating anti-gibberellins like ancymidol or paclobutrazol. Environmental control: increasing light intensity (PPFD > 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and ensuring good airflow around culture vessels. Adding osmotic agents like mannitol or sorbitol to the medium can also help reduce water potential (Hoque, 2010; Debnath et al., 2019).

Challenge 5: Efficient Rooting and Acclimatization

Achieving high rates of robust root development *in vitro* is essential, but sugarcane microshoots can be recalcitrant, producing weak or callused roots. The subsequent transition from the heterotrophic, high-humidity *in vitro* environment to autotrophic, variable greenhouse/field conditions (acclimatization/hardening) is a major bottleneck, often associated with high mortality due to desiccation, shock, or disease. Strategies include optimized rooting media: using half-strength MS salts, specific auxins (IBA is generally superior to NAA at 1.0-3.0 mg/L), and sometimes activated charcoal. Pulse treatments with high auxin concentrations (e.g., 100-250 mg/L IBA for 1-24 hours) followed by transfer to auxin-free medium often yield superior root systems with less callus. Ex vitro rooting: transferring unrooted microshoots directly to sterile, well-drained substrates (e.g., cocopeat:perlite) under high-humidity conditions (mist/fog) with or without auxin dip. For acclimatization, gradual humidity reduction using mist/fog systems, humidity tents, or shaded polyhouses over 2-4 weeks is critical. Nutrient support with diluted, balanced fertilizers and disease prevention using fungicides/drenches are essential. In vitro hardening techniques like increasing light intensity, sucrose reduction, or using CO_2 enrichment in the late *in vitro* stages can prime plantlets for transition. Utilizing biological agents like mycorrhizal fungi or plant growth-promoting rhizobacteria (PGPR) during potting enhances root establishment and stress tolerance (Sreedhar et al., 2008; Tiwari et al., 2011; Chavan et al., 2021).

Challenge 6: Achieving Cost-Effective Scale-Up and Automation

Commercial viability hinges on reducing production costs per plantlet while maintaining quality. Manual handling is labor-intensive and expensive. Solutions involve automation: implementing robotic systems for media dispensing, explant inoculation, and plantlet handling. Liquid culture systems: utilizing bioreactors, particularly Temporary Immersion Systems (TIS) like RITA® or SETIS™, which dramatically increase multiplication rates, reduce labor, improve nutrient/gas exchange, and can lower hyperhydricity compared to semi-solid cultures. Optimization of media components: reducing agar concentration, substituting expensive reagents, using cost-effective carbon sources like table sugar, and optimizing vessel size/fill volume. Efficient facility design: maximizing space utilization with vertical racks, optimizing environmental control systems for energy efficiency, and implementing strict workflow protocols to minimize contamination losses and rework. Integration with nursery operations: streamlining the transfer from *in vitro* to greenhouse hardening and pre-field nursery stages (Alvard et al., 1993; Akdemir et al., 2014; Srivastava et al., 2016).

Challenge 7: Ensuring Consistent Quality and Phytosanitary Status

Maintaining the genetic identity, health, and vigor of millions of plantlets is paramount. Undetected somaclonal variants or pathogen breakthroughs can have catastrophic commercial consequences. Robust Quality Assurance/Quality Control (QA/QC) systems are essential. This includes molecular genetic testing at different stages to monitor clonal fidelity. Pathogen indexing: employing sensitive techniques like PCR, ELISA, or next-generation sequencing (NGS) to detect known systemic pathogens (viruses, bacteria, phytoplasmas) in source plants, culture stocks, and hardened plants. Vigor assessment: standardized protocols for evaluating rooting percentage, shoot height, leaf development, and survival rates during acclimatization. Traceability systems: meticulous record-keeping linking every plantlet batch back to the original mother plant and culture conditions. Adherence

to certification standards set by national plant protection organizations is critical for market access (Rott et al., 2000; Ramdoyal et al., 2019; Chavan et al., 2021).

Conclusion

Commercial-scale micropropagation of sugarcane is not merely scaling up a lab protocol; it requires integrated solutions addressing intertwined biological, technical, and economic challenges. Success hinges on:

Robust Initiation: Mastering contamination and phenolic issues through advanced sterilization, endophyte management, and antioxidants.

Stable Multiplication: Utilizing axillary bud proliferation pathways, optimizing media and environment to minimize hyperhydricity and somaclonal variation, and employing TIS bioreactors for efficiency.

Effective Rooting and Acclimatization: Implementing pulse treatments, *ex vitro* rooting, and sophisticated hardening protocols with biological support.

Rigorous QA/QC: Enforcing genetic fidelity testing, pathogen indexing, and vigor assessment throughout the process.

Cost Optimization: Leveraging automation, bioreactors, efficient facility design, and workflow optimization.

While significant progress has been made, continuous research into understanding the molecular basis of somaclonal variation, developing more sensitive pathogen detection methods, refining bioreactor designs, and discovering cost-effective media substitutes remains crucial. By systematically implementing these strategies, micropropagation can deliver on its promise: providing sugarcane industries globally with abundant, high-quality, disease-free planting material, leading to substantial yield increases and sustainable productivity gains.

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