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Low cost, semi-automated hybrid bioreactor system for clean plant production of horticultural crops

Temporary immersion system (TIS) bioreactors for micropropagation of fruit crops have emerged as an ideal choice due to reduced production costs, rapid biomass accumulation, substantially higher multiplication rates, prevention of hyperhydricity through improved gas exchange, and ease of automation. This paper provides a detailed overview of micro-propagation of fruit crops using different TIS bioreactors.

Keywords: Bioreactor-mediated propagation, Clean plant production, Micropropagation, Nutri-smart plantlets, Temporary Immersion System

MICROPROPAGATION has become crucial for providing quality plants to meet the demand for commercial cultivation of horticultural crops. It facilitates the rapid bulking up of plants in small spaces, making it a suitable method for mass multiplication. Micropropagation has assumed substantial commercial importance across the globe, and many fruit crops are being produced at an industrial scale (banana, papaya, pineapple, apples, and date palm) through conventional tissue culture techniques. Despite some fruit crops being multiplied at industrial scale, the majority of woody, perennial fruit crops are recalcitrant to tissue culture. Micropropagation protocols are available, but their exploitation at a commercial scale is still awaited for crops such as mango, guava, pomegranate, coconut, litchi, cashew, and several others. Most protocols are genotype-specific and cannot be utilized for different genotypes or cultivars of the same species. A vast literature on micropropagation protocols of fruit crops exists, which may be repeatable at the lab scale. However, most protocols cannot be exploited commercially due to lack of parameterization of several important factors, such as age of the stock plant, time of explant collection, physiological stage of the explant, and status of the inborn microbial load of the stock plant.

Conventional tissue culture techniques usually involve small glass bottles filled with nutrient medium gelled with solidifying agents (most commonly agar), onto which plant tissue is inoculated for further growth and development. These techniques require transferring plant tissue between bottles containing nutrient media fortified with various plant growth hormones for biomass enhancement. This process is highly labour-intensive, requiring a large number of skilled personnel. Manpower alone accounts for 6–70% of the cost of tissue culture–propagated plants, while gelling agents contribute 10–20% of the medium cost. Lack of skilled manpower is another bottleneck limiting the expansion of this industry on par with other knowledge-based industries. Several bioreactor systems have been developed over time and tested for micropropagation of horticultural crops. There is a need to establish less labour-intensive, more automated tissue culture technologies to make the process more cost-effective. Bioreactor-mediated micropropagation can address these challenges. This paper describes advances in the development of bioreactors for tissue culture of banana and bioreactor-mediated in vitro regeneration of papaya as a potent technology for clean plant production programs in India.

Clean plant production of banana through bioreactor

Conventional tissue culture is increasingly being replaced by automated bioreactor systems due to improved gas exchange, higher biomass accumulation per subculture, reduced somaclonal variation, and a significant reduction in production costs. Tissue culture technology is now exploited at an industrial scale for mass multiplication of banana. However, most industries still rely on conventional semi-solid tissue culture systems for banana propagation due to the lack of robust temporary immersion bioreactor systems.

We have designed, developed, and validated a double-decker bioreactor system for the production of wilt-tolerant (Foc TR4) banana plantlets using in vitro bio-immunization technology. In vitro bio-immunization is a novel tissue culture approach for producing Fusarium wilt–tolerant banana plants. This process involves introducing the biomolecule into banana tissue culture plantlets during the in vitro organogenesis phase.

Field studies conducted in Fusarium wilt hotspot regions of Ayodhya district (Uttar Pradesh) and Katihar district (Bihar) revealed that bio-immunized plantlets were able to reduce disease incidence by approximately

TIS technology	Material used	Power source	Purpose
RITA	Polypropelene	Pneumatic and gravity	Intensive in vitro propagation of diverse crops
BIB (Bioreactor of immersion by bubbles)	SS & Glass	Pneumatic and gravity	Micropropagation of Oncidium orchid
Twin Flask	Glass	Pneumatic	Micropropagation of carnation and banana
Hybrid Ebb and flow	Glass	Pneumatic and gravity	Cultivation of high-density hairy root cultures
Rocker bioreactor	Polycarbonate	Mechanical	Micropropagation of carnation
Rotating drum	Glass or plastic	Mechanical	Micropropagation of potato
Plantform	Polypropelen	Pneumatic	Micropropagation of apple, banana, raspberry, blackberry, strawberry, date palm, citrus, and olive
SETIS	Polypropelen	Pneumatic	Micropropagation of pineapple, banana, strawberry, and date palm

Micropropagation of banana under TIS bioreactor

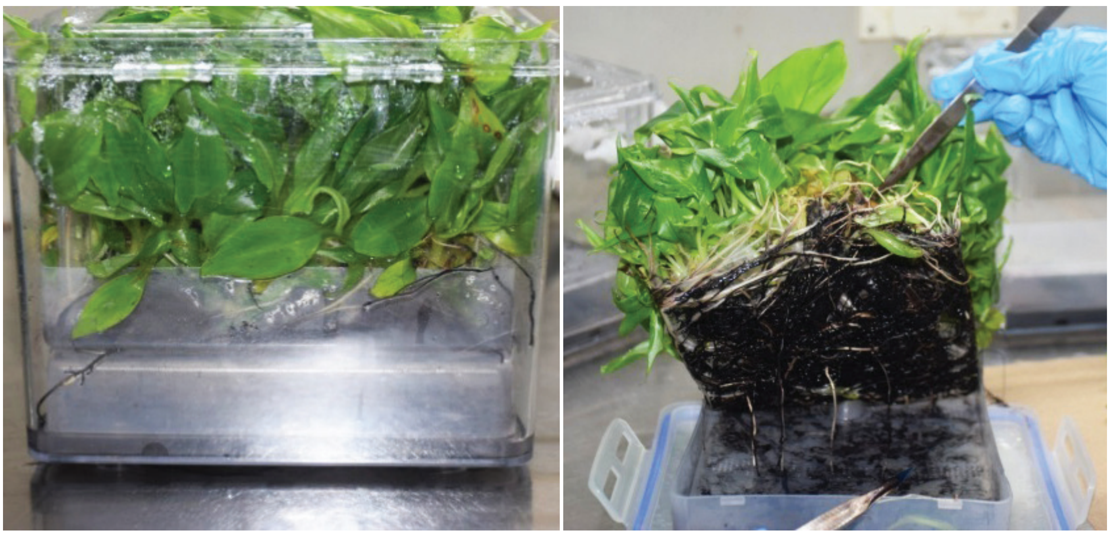
98% for 9 months after planting and by 85% until harvest. Banana shoot clumps immersed for 3 minutes every 6 hours exhibited a threefold higher multiplication rate compared to conventional tissue culture systems. Biomass accumulation during the subculture cycle was also significantly higher in bioreactors than in semi-solid systems.

Plantlets produced through both semi-solid systems and temporary immersion bioreactors were analyzed for genetic fidelity using ICAR-IISR primers and were confirmed to be genetically true-to-type. Field evaluation of banana plantlets from both systems showed no significant difference in yield under Lucknow conditions.

Clean plant production of papaya through bioreactors

In vitro regeneration systems in papaya (*Carica papaya* L.) were standardized using a temporary immersion bioreactor system (DDTIB). Immature seeds were harvested from selfed fruits of the papaya variety Pusa Delicious and cultured following the methodology of Mishra et al. (2007). Somatic embryogenesis was achieved on ½ MS medium supplemented with 2,4-D (10 mg/L), glutamine (400 mg/L), and sucrose (60 g/L) under a conventional in vitro system.

The matured embryos were multiplied in a temporary immersion bioreactor using a 2 minute immersion frequency at 8 hr intervals. While six shoots per clump were obtained in the semi-solid system, a higher multiplication rate of nine shoots per clump was achieved in the temporary immersion bioreactor. The shoots were subsequently rooted on ½ MS medium fortified with 2 mg/L IBA under the same bioreactor conditions, with a 2 minute immersion every 8 hours.



Production of bio-immunized banana cv. G-9 through bioreactor

CONCLUSION

A successful micropropagation system was developed for banana and papaya using temporary immersion bioreactors. Most fruit crops are woody and perennial in nature, and the majority of subtropical fruit crops are recalcitrant to tissue culture.

Semi-solid tissue culture systems do not expose sufficient explant surface for nutrient absorption, leading to poor shoot proliferation and limited biomass accumulation. Bioreactor systems promote automation, thereby reducing the cost of production. Additionally, the reduced use of gelling agents further lowers production costs at a commercial scale. Bioreactor-mediated propagation can facilitate large-scale production of recalcitrant fruit crops such as mango, guava, pomegranate, litchi, cashew, and coconut. Efforts are currently underway to regenerate mango, guava, and jamun through bioreactor systems.

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