DOI : 10.58993/ijh/2024.81.1.1

Indian J. Hortic. 81(1), March 2024: 1-10

New paradigm shifts in micropropagation of fruit crops through bioreactors - a review

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ABSTRACT

Due to their distinct advantages of reduced production costs, high proliferation rate, high biomass index within relatively shorter periods, as well as significant reductions in hyperhydricity in plants as a result of efficient gas exchange, oxygen supplementation and automation, bioreactors, specifically temporary immersion systems (TIS), are being utilized for mass multiplication of forestry and horticultural crops. In tissue culture of banana, date palm, strawberrys, papaya, citrus, grape, pineapple, apple, pear, plum, chestnut, pistachio nut, apricot, sweet cherry, and almond, a variety of TIS bioreactors were used, including RITA, Plantform, SETIS and twin glass airlifts. TIS Bioreactors need to be improved in terms of space utilization. The space utilization was found to be highest with the Plantform system (80%) and lowest in the Twin Flask system (26%). Higher head space provides better plant growth and lesser fogging on the walls of the bioreactor. Most bioreactors have not been designed to facilitate better root production *in vitro*. Roots get coiled and cluttered, which needs improvement in design. The provision of illumination in each tank will facilitate better morphogenesis. This paper describes the micropropagation of fruit crops using different TIS bioreactors.

Key words: Bioreactor, BIB, Continuous immersion system, Fruit crops, Micropropagation, Plantform, RITA, Temporary immersion system, TIB.

INTRODUCTION

Micropropagation has become crucial for providing quality plants to meet the demand for commercial cultivation of horticultural crops. Micropropagation facilitates the rapid bulking up of plants in small spaces, making it a suitable method for mass multiplication (Sharma *et al.*, 50). Micropropagation has assumed substantial commercial importance across the globe, and many fruit crops (banana, papaya, pineapple, apple, and date palm) are being produced at an industrial scale through conventional tissue culture techniques. Despite the fact that some of the fruit crops are being multiplied through tissue culture at industrial scale, however, majority of woody, perennial fruit crops are recalcitrant to tissue culture. Micropropagation protocols are available but their exploitation at commercial scale is still awaited such as mango, guava, pomegranate, coconut, litchi, cashew, Indian gooseberry, *bael* and several other fruit crops (Mishra *et al.*, 41; Mishra *et al.*, 42; Mishra *et al.*, 43; Mishra *et al.*, 44; Sharma *et al.*, 50; Damodaran *et al.*, 19). Most micropropagation protocols are genotype-specific and cannot be utilized for different genotypes/cultivars of the same species. A vast literature on micropropagation protocol of fruit crops exists, which may be repeatable at the lab scale (Carlo *et al.*, 16; Carlos *et al.*, 17; Corona *et al.*, 18). However, most of the protocols could not be exploited at a commercial scale due to lack of parameterization of several important factors such as age of stock plant, time of collection of explant, physiological stage of explant, status of inborn microbial load of stock plant *etc*. Conventional tissue culture usually involves small glass bottles filled with nutrient medium gelled with solidifying agents (most commonly agar-agar) upon which plant tissue is inoculated for further growth and development. Conventional tissue culture techniques warrant the transfer of plant tissue in small bottles containing nutrient media fortified with differential concentrations of plant growth hormones for biomass enhancement. This requires employing large number of skilled human resources to complete the process, which makes tissue culture a highly labour intensive industry. Manpower alone accounts for 6-70% of the cost of tissue culture propagated plants. Gelling agents make up 10-20% of the cost of culture medium (Nagori *et al.*, 45).

Lack of skilled manpower in tissue culture is another bottleneck in the expansion of this industry at par with other knowledge-based industries. There is a need to establish less labour-intensive, more automated tissue culture technology to make it more cost-effective. Bioreactor-mediated micropropagation technology can address the aforementioned issues. This paper describes the advances made in developing bioreactors for tissue culture, the problems faced

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in optimising bioreactor technology, and possible interventions to make it more viable with special reference to fruit crops.

WHAT IS BIOREACTOR?

Bioreactors were developed with aim of culturing microorganism, which were later utilized for secondary metabolite production. Bioreactor is a vessel of varying size that provides optimum growth condition by regulating chemical and physical parameters. It also regulates the availability of air and nutrient medium exposure to plant tissue. Two types of bioreactors exist: temporary immersion system (TIS) and continuous immersion system (CIS). TIS and CIS can be utilized depending on the plant species in question or the targeted growth stage. TIS employs temporary immersion of explants and provides a pathogen-free environment suitable for clonal multiplication under *in vitro* conditions. This technology offers higher biomass production in comparatively less time, saving the cost of gelling agent, minimal human footprint and ease of operation control.

In contrast to conventional tissue culture system (Semi-solid tissue culture), this system allows for more surface area of explants in contact with nutrient medium leading to more biomass production. Efficient input utilization reduces production costs, rendering it the most cost-effective micropropagation system. CIS employ immersion of whole explants, which in some species leads to hyperhydricity and abnormal plant development due to scarcity of air in the liquid medium, which augments oxidative stress and leads to tissue necrosis. The problem can be circumvented by providing air through agitation or aeration. Recently, a huge surge of interest in TIS bioreactor has led to its commercialization.

TEMPORARY IMMERSION SYSTEM (TIS) BIOREACTOR

TIS bioreactors have travelled a long journey from lab curiosity to commercial adoption. The use of bioreactors in plant propagation was initially reported in 1981 for begonia culture by Takayama and Akita (54). Tisserat and Vandercook (56) probably were the first to design an Automated Plant Tissue Culture System (APCS). A standard design includes two jars (plastic or glass), one for the liquid medium and the other for the cultures. Another type of temporary immersion bioreactor is a system in which a single vessel with a reservoir on one side is mechanically tilted at regular intervals (Adelberg and Simpson, 4). In this manner, 48 media regularly batches the cultures in the vessel, keeping the propagules upright. Other temporary immersion bioreactors have different vessel designs and rotations. As the tank rotates, the culture is periodically immersed in the medium. Temporary immersion bioreactors are simple and inexpensive to run. As a result, several commercial micropropagation laboratories have begun to include these devices in their production protocols. TIS bioreactors are advantageous as they reduce hyperhydricity, reduce consumption of consumables, reduce energy and labour requirements, stop the accumulation of ethylene and CO_2 in the vessels and augment shoot proliferation and plant growth due to more exposure of explants with liquid medium (Escalona *et al.*, 22; Aitken and Davies, 6). Efforts have been made to automate bioreactor with a computer and software. A glass container was connected to an oil-free compressor and additional equipment and then to a computer *via* an RS232 connection. The software was created utilizing PHP and a MySQL database structure (Kana *et al.*, 34). However, there is a need to use Artificial Intelligence (AI) with TIS technology to reduce human interventions and achieve efficiency. A detailed review of TIS bioreactors has been reported by many authors (Watt, 59; Georgeive *et al.*, 27; Georgeive *et al.*, 28; Sanchez and Vidal, 51; Mirzabe *et al.*, 40). Development of some of the TIS technology, along with the material used, is described in Table 1 and Fig. 1.

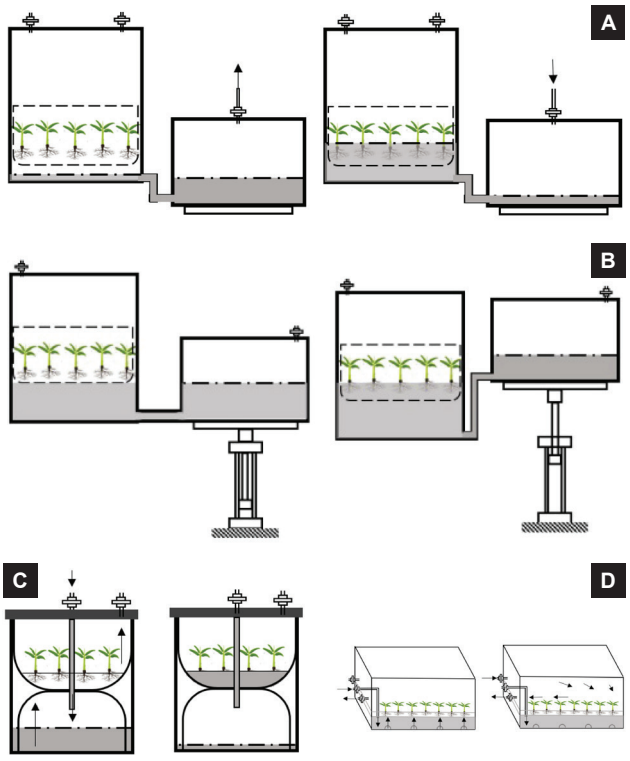

Fig. 1. Different types of TIS bioreactors: Twin Flask Bioreactor (A), Twin Flask Bioreactor with mechanical leg (B), RITA (C) and Plantform (D).

Table 1. Different types of TIS bioreactors used in micropropagation.

TIS technology	Material(s) 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 *Bambusa vulgaris*
Hybrid Ebb and flow	Glass	Pneumatic and gravity	High-density cultivation of hairy root cultures
Thermo-photo bioreactor	Pyrex glass	Pneumatic and gravity	Micropropagation and secondary metabolite synthesis of Antarctic hair grass
Rocker bioreactor	Polycarbonate	Mechanical	Micropropagation of carnation
BioMint	Polycarbonate	Mechanical	
Rotating drum	Glass or plastic	Mechanical	Micropropagation of potato
Box in Bag	Polycarbonate, polyethylene and nylon	Pneumatic and gravity	Micropropagation of diverse crops

TIS TECHNOLOGY IN MICROPROPAGATION OF FRUIT CROPS

Several fruit crops are multiplied through tissue culture technology, such as banana, date palm, strawberry, pineapple *etc*. Tissue culture technologies of several fruit crops are available. However, the same has not been exploited for TIS technology. Here, we describe the development of temporary immersion bioreactor-mediated micropropagation in some fruit crops (Table 2).

BANANA (*MUSA* SPP.)

Banana is multiplied using tissue culture technology worldwide. This is one of the most micropropagated fruit crops across the globe. *In-vitro* micropropagation protocol was standardized and utilized for several banana cultivars (Cronauer and Krikorian, 14; Madhulatha *et al.*, 39). Shoot tip culture was the most popular and common for propagation among different tissue culture techniques. The semi-solid system of tissue culture is labor-intensive and time-consuming. TIS technology can effectively replace semi-solid TC technology of bananas (Fig. 2). Farhani and Majd (24) studied TIS technology for micropropagation of banana cultivar Dwarf Cavendish in 2012 and they found that TIS enhanced shoot numbers to the tune of 7 which was significantly higher

Table 2. Application of TIS technology for micropropagation of certain fruit crops.

Crop	Bioreactor used	Explant(s)	Reference(s)
Banana	Glass tank fitted with aeration, SETIS,	Shoot	Farhani and Majd (24), Bello-Bello *et al.* (11), Abdulmallik *et al.* (2), Uma *et al.* (57)
Date palm	Plantform, RITA	Immature inflorescence	Othmani *et al.* (46), Almusawi *et al.* (9), Alkhateeb and Alturky (8), Fki *et al.* (26), AlKhayari and Naik (5)
Strawberry	RITA	Shoot tip, leaf	Hanhineva *et al.* (32), Debnath (21), Hawang *et al.* (33)
Citrus spp.	RITA, NALGENE	Somatic embryos	Cabasson *et al.* (13), Perez and Debergh (47)
Grape	TIB-1, TIB-2	Shoot apices	Kryukov *et al.* (37)
Apple	RITA, Airlift bioreactor, Ebb and Flood	Shoot bud	Chakrabarty *et al.* (15), Kim *et al.* (36)
Pear		Shoot bud	Arruda *et al.* (10)
Plum	RITA	Shoot	Godoy *et al.* (29)
Chest nut	Plantform, RITA	Shoot tip	Vidal *et al.* (58)
Pistachio nut	RITA	Nodal bud	Akdemir *et al.* (3), Tilkat *et al.* (55)
Apricot	TIB	Shoot bud	Khafri *et al.* (35)
Sweet cherry	TIB	Shoot	Godoy *et al.* (29)
Almond	TIB	Shoot	Ebrahimi *et al.* (23)

Fig. 2. Micropropagation of banana in Plantform bioreactor (Courtesy: M. Mishra, CISH, Lucknow).

than other methods. Bello *et al.* (11) noticed that the SETIS™ bioreactor was more efficient and resulted in better development in the plants obtained *in vitro* and *ex vivo* of the banana cv. Grand Naine. This system proved to be an excellent choice for banana micropropagation. Abdulmalik *et al.* (2) established protocol for micropropagation of banana in Nigeria using TIS technology. Uma *et al.* (57) described a cost-effective TIS system of micropropagation for Rasthali, a banana cultivar in India. 250 ml glass tank TIS having provision of 2 min. medium immersion after 6 hours found effective for shoot proliferation and rooting in banana. The production output of 1496 ± 110 shoots/TIS was obtained after 6th cycle. A multiplication rate of 1:2.7 was achieved under TIS technology. The plants obtained through TIS were genetically stable and physiologically similar to plants obtained through semi-solid tissue culture system.

PAPAYA (*CARICA PAPAYA* L.)

Papaya is cultivated in tropical and subtropical countries of the world. Micropropagation through somatic embryogenesis (Fitch *et al.*, 26; Mishra *et al.*, 42; Fitch and Manshardt, 25) or through nodal shoot (Litz and Conover, 38) has already been worked out. However, Coronaa *et al.* (14) propagated papaya hybrid MSXJ in a bioreactor (SETIS and BIT) using nodal shoots of papaya. Two min. immersion frequency of MS medium fortified with 2.2 µM BAP + 0.2 µM NAA after every 8 h gave a shoot proliferation rate of 15.06 shoots in SETIS system followed by 9.8 shoots in BIT bioreactor system. Gomez and colleagues successfully cultured axillary shoots of papaya in TIS, achieving a 100% survival rate without oxidation. An inoculum density of 8 was immersed for 2 min. every 6 h, resulting in a multiplication rate of 5.05±0.06, much higher than the rate seen in the semi-solid system (1.20±0.02). Posada-Pérez *et al.* (48) developed a technique for somatic embryogenesis in papaya utilizing RITA. About 200 mg fresh somatic embryos of papaya cv. Mardola Raja gave rise to 95% plantlets when incubated in TIS having 0.02 µM BAP and 2.90 µM GA_3. Papaya is an important fruit crop in terms of its regeneration and from a transgenic development point of view. There is a need to work out the repeatable *in vitro* regeneration and genetic transformation in papaya under bioreactors.

DATE PALM (*PHOENIX DACTYLIFERA* L.)

Date palm is one of the most important fruit crops for nutritional security. The date palm is a dioecious fruit crop with distinct male and female plants. Micropropagation of female plants assumes great significance for the supply of quality planting material. The cost of tissue cultured date palm plants needs to be reduced drastically. The cost of date palm tissue-cultured raised plants is 100 times more than that of tissue-cultured banana plantlets (Rajmohan, 49). This led to huge interest among researchers and the private sector to explore TIS technology for date palm. There are contradictory reports in date palm as to which system is better. Othmani *et al.* (46) detailed the use of TIS micropropagation for date palm regeneration through shoot organogenesis, resulting in a notable increase in multiplication rate (5.5 times higher than semi-solidified tissue culture) and improved plant material quality, leading to reduced production costs. Almusawi *et al.* (9) utilized a Plantform bioreactor for date palm micropropagation and they found an efficient multiplication rate, enhanced biomass in less time and uniform development of somatic embryos of date plam under TIS technology. However, Plantform bioreactors tested for date palm varieties, *viz*., Medjhool and Boufeggous revealed that the number of shoots and roots was slightly higher in the semi-solid system than in TIS (Abahmane, 1). Similar results were obtained by Alkhateeb and Alturky (8) who also noted that the solid system performed better in terms of shoot proliferation. However, Fki *et al.* (26) and Al Khayari and Naik (5) opined that TIS technology augmented shoot proliferation in date palm compared to semi-solid systems.

PINEAPPLE (*ANANAS COMOSUS* L.)

Pineapple is another fruit crop where microproapagation technology has been exploited commercially to supply quality planting material. Conventional tissue culture technology is being used to micropropagate pineapple across the globe. However, González-Olmedo *et al.* (31) were the first to report TIS technology in pineapple propagation program Later, Escalona *et al.* (22) compared the TIS technology with CIS and semi-solid TC technology in pineapple and found that it enhanced shoot proliferation significantly. Ten litre TIS bioreactor was utilized to proliferate *in vitro* shoots in MS medium fortified with BAP 2.1 mgl^{-1} + NAA 0.3 mgl^{-1} + paclobutrazol 1 mgl^{-1}. As many as 191.8 plants/l were obtained through TIS technology in 4 weeks. Pineapple shoots micropropagated on TIS showed a high sugar and nitrogen uptake increase. It was revealed that pineapple shoots relied more on culture medium than photosyenthesis for food. The dry weight and leaf area were higher in TIS-propagated plants (Escalona *et al.*, 22). Scheidt *et al.* (52) compared the Bioreactor of Immersion by Bubbles (BIB) and Reactor of Temporary Immersion (RITA), having an immersion frequency of 2 hours for 15 min. with liquid TC system in 200 ml flask. MS medium fortified with BAP 1 mgl^{-1} + NAA 0.25 mgl^{-1} + sucrose 30 gl^{-1} + Tween-20 0.5 µL. BIB gave 3-fold higher multiplication rate of pine apple than RITA and conventional TC system. A study by Ayenew *et al.* (7) revealed that pineapple explants cultivated on TIS with full-strength MS medium supplemented with 2 mgl^{-1} IBA and 30 gl^{-1} sucrose showed improved results, yielding an average multiplication of 13.17 shoots per explant within six weeks of culture. Plantlets grown on TIB with half-strength MS medium containing 3 mgl^{-1} IBA and 40 gl^{-1} sucrose produced an average of 16.33 roots, each measuring 6.27 cm in length, with well-developed hairy roots within four weeks. These plantlets showed improved performance during acclimatization and in open field conditions. Solórzano-Acosta *et al.* (53) studied the design parameter of TIS technology for pineapples in Peru and built an indigenous TIS system with 24 tanks with 2 l each capacity. The parameters, such as duration of propagation cycle, immersion frequency, duration of aeration *etc*, were standardized for micropropagation of pineapple cv. Trujillana Red. The multiplication rate in TIS was 6.5 times per propagative unit inoculated in 30 days.

STRAWBERRY (*FRAGARIA × ANASSA* DUCH.)

Strawberry is one of the most commercial berry crops across the world's temperate and subtropics region. Micropropagation of strawberry started around two decades ago. Boxus (12) reviewed the research on micropropagation of strawberry. However, tissue culture plants cost much higher than plants produced through runners. This led to exploration of TIS technology in strawberry. Five commercial cultivars of strawberry, *viz*., Bounty, Jonsok, Korona, Polka, and Zephyr, were propagated in RITA and compared with a semi-solid tissue culture system in a regeneration medium. TIS gave a regeneration frequency of 70 ± 8 to 94 ± 2%, whereas semi-solid system gave a regeneration frequency of 83 ± 5 to 92 ± 3% (Hanhineva *et al.*, 32). RITA was utilized for micropropagation of strawberry var. Bounty using immersion frequency of 15 min. after every 4 h. Hyperhydricity was induced in a medium containing TDZ. However, replacing TDZ with zeatin solved the problem (Debnath, 21). Semi-solid system of micropropagation was compared with TIS in strawberry and raspberry fruit crops. The multiplication rate enhanced from 1.9 in the conventional system to 4.1 in TIS in strawberry, whereas a multiplication rate of 3.2 under the conventional system was improved to 6.7 in TIS in raspberry (Georgieva *et al.*, 27). A recent study compared traditional and temporary immersion system (TIS) methods for micropropagation of strawberry plants. Results showed that plants grown in TIS culture had the best development, with 1.10 g of fresh weight and 0.17 g of dry weight per explant. The growth and proliferation rate of plantlets cultivated in TIS was believed to be better than those grown in semi-solid or liquid culture. TIS has the main advantage of removing volatile chemicals like ethylene by enhancing ventilation in the culture vessel by forced aeration (Hawang *et al.*, 33). Kryulov *et al.* (37) found that integrating a semi-solid system for 4 weeks followed by micropropagation in a bioreactor enhanced shoot proliferation of strawberry cv. Murano to the tune of 20-fold.

CITRUS SPP.

Citrus has been researched vigorously from micropropagation point of view. However, reports on temporary immersion system-mediated regeneration are scanty. Cabasson *et al.* (13) devised a protocol for the growth of somatic embryos of *C. deliciosa*. TIS promoted 66% of citrus embryos into cotyledonary stage and hampered secondary embryogenesis at the time of germination. TIS (RITA & NALGENE) mediated regeneration of *C. aurantium* and *C. sinensis* somatic embryos revealed that 15 min. immersion frequency after every 12 h enhanced embryo production in RITA (Perez and Debergh, 47).

GRAPE (*VITIS VINIFERA* L.)

The application of bioreactors in grape micropropagation has recently been attempted. *In vitro* propagation of grape cv. Traminer Pink was initiated on semi-solid medium then advanced to temporary immersion system using TIB-1 and TIB-2 for 6 weeks. The multiplication rate in grape using integrated system was more than 5-fold compared to the control. *In vitro* rooting in bioreactor required less IBA. TIB-1 produced 55.8 ± 5.3 shoots, while TIB-2 produced 60.6 ± 7.5 shoots. In TIB-1, the shoot biomass grew from 30 to 1000 mg, whereas in TIB-2, it rose to 1600 mg. However, rooting under a bioreactor needs further improvement in grape (Kryukov *et al.*, 37).

TEMPERATE FRUIT CROPS

Efforts have been made to utilize TIS for micropropagation of temperate fruit crops such as apple, pear, plum, apricot, chestnut, pistachio nut and sweet cherry. Chakrabarty *et al.* (15) propagated apple rootstock M-9-EMLA using Ebb and Flood Air Lift Balloon Bioreactor (BTBB) by immersing nodal explants 4 times daily for 15 min. each. Kim *et al.* (36) later showed that an airlift bioreactor with an immersion frequency of 3 h may produce more virus-free apple plants in a shorter time than traditional culture systems. Furthermore, secondary xylem was well formed in the stems of plants cultivated using the temporary immersion approach. This technique has great potential for growing robust plants appropriate for acclimatization. Tissue culture techniques were also used for the multiplication of pear trees. Arruda *et al.* (10) assessed three pear rootstocks and determined that TIS is superior to the traditional technique for propagation. Pear showed the highest multiplication rate of 24 shoots when grown in temporary immersion bioreactors with salt-enriched medium. In contrast, the lowest rate of 4 shoots was seen in solid medium with a typical MS composition. Hyperhydricity was not found in any of the pear cultures. The plum (*Prunus domestica* L.) was cultured in a RITA bioreactor for propagation. The multiplication rate was twice as high as that of the semi-solid system, and the shoot length was found to be 1.7 times greater in TIS technology (Godoy *et al.*, 29). Vidal *et al.* (58) assessed the RITA and Plantform bioreactors for micropropagation of hybrid chestnut and compared them with the semi-solid system. The plants obtained from liquid systems were modest, but the rate of multiplication and cost of production was much higher. RITA proved suitable for micropropagation of pistachio nut and its rootstocks utilizing nodal bud explant in MS medium supplemented with 4 mg/l BA and 0.1 mg/l GA_3. The semi-solid system caused necrosis in shoots, but shoots in the TIS were healthy (Akdemir *et al.*, 3). Tilkat *et al.* (55) developed a method for mass shoot production of *Pistacia khinjuk* stocks utilizing TIS technology. The TIS system was found to enhance shoot proliferation with a shoot forming capacity index of 3.12 and decrease shoot hyper-hydricity by 8% when immersed for 10 min. every 16 h. Compared to traditional solid culture methods, apricot can be efficiently micropropagated in Temporary Immersion Systems (TIS). Using a temporary immersion bioreactor system for 10 min. per hour significantly increased the number of lateral shoots produced, with improved shoot quality, across all tested apricot cultivars. The QL medium with half the normal strength and 19.68 µmol l^{-1} of IBA produced the highest percentage of root formation in all apricot cultivars studied (Khafri *et al.*, 35). The sweet cherry, scientifically known as *Prunus avium* L., is a significant fruit crop. Research has shown that using TIS technology, it can propagate sweet cherries and their rootstocks. The TIS system for sweet cherry types and cherry rootstocks is a viable, more efficient, and quicker propagation method than solid medium culture. Plants grown by TIS exhibit a limited autotrophic potential after 14 days of cultivation (Godoy *et al.*, 29). The almond cultivar Shahroodi was initially rid of viruses using thermotherapy. The virus-free explants were grown in TIS with 1 mgl^{-1} BAP, 0.5 mgl^{-1} GA3, 0.01 mgl^{-1} IBA, and 3% sucrose. They were then planted in half-strength MS medium with 1 mgl^{-1} IBA and 0.5 mgl^{-1} IAA. Fresh weight of almond shoots increased to 29.66 mg in TIS compared to 8.71 mg in the SS system (Ebrahimi *et al.*, 23).

INTEGRATION OF BIO-IMMUNIZATION TECHNOLOGY WITH BIOREACTOR

A novel method was developed and validated for infusing a metabolite-based biomolecule (Bio-immune) during the *in vitro* stage of banana cv. Grand Naine in order to produce *Fusarium oxysporum* f.sp. *cubense* TR4 tolerant plantlets (Damodaran *et al.*, 19). The new bio-immune mixture showed strong antifungal activity against *Foc* TR4, achieving 100% inhibition at a 2.5% concentration on the 5th, 7th, and 9th days after inoculation. Bio-immune integration occurs at the *in-vitro* shoot proliferation stage in banana cv. Grand Naine showed a marked improvement in the growth of both roots and shoots. The medium fortified with 0.5% bio-immune produced 12.67 shoots per clump, while the control only produced 9.67. The bio-immune plants had a considerably greater maximum root number of 7.67 compared to the

control group with 5.0 roots. During acclimation, bio-immunized banana transplants had a higher survival rate of 97.57% compared to the control group's rate of 94.53%. In the ever-evolving field of fruit crop micropropagation, the emergence of Temporary Immersion Bioreactors (TIS) shines as a beacon of innovation, poised to redefine the tissue culture plant production landscape. The review paper discusses the various advantages of TIS, focusing on two key aspects: the use of Bio-immunization Technology for disease control in tissue culture and the importance of bioreactors in producing bio-immunized plants on a large scale.

A groundbreaking application explored in this context is the incorporation of Bio-immunization Technology within TIS, specifically addressing the challenge posed by TR-4-induced wilt disease in banana crops (Damodaran *et al.*, 19). This inventive approach not only demonstrates the adaptability and responsiveness of TIS bioreactors to real-world challenges but also unveils promising avenues for sustainable disease management in the commercial cultivation of fruit crops. The study emphasizes the banana as a prime example, illustrating its potential to serve as a model for similar integrations to combat diseases through bio-immunization in other fruit crops.

CONCLUSION

Most of the fruit crops are woody perennial. Several temperate, tropical and subtropical fruit crops are recalcitrant to tissue culture. A semi-solid tissue culture system does not expose enough explant surfaces for absorption of nutrient medium, leading to poor shoot proliferation and biomass accumulation. Several authors have demonstrated that difficult to micropropagate fruit crops such as chestnut, pistachio nut and apricot have performed considerably well in bioreactors. Bioreactor systems promote automation, thereby reducing the cost of production. Cost of the gelling agent itself reduces the cost of production at a commercial scale. Bioreactor-mediated propagation can help in the large-scale production of some recalcitrant fruit crops like mango, guava, pomegranate, litchi, cashew, coconut, *etc*. In the broader context of large-scale plant production, the review underscores the pivotal role assumed by bioreactors in orchestrating the bio-immunization process. The bioreactor plays a crucial role in micropropagation by providing accuracy, scalability, and enabling the successful culture of bio-immunized plants on a large scale. This paradigm shift in the utilization of TIS and bio-immunization technologies hold significant implications for the advancement of sustainable and efficient fruit crop propagation practices.

DECLARATION

The authors declare that they do not have any conflict of interest.

ACKNOWLEDGEMENT

The authors express gratitude to the Director of ICAR-Central Institute for Subtropical Horticulture, Lucknow for supporting the work.

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Received : February 2024; Revised : March 2024;
Accepted : March 2024