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Review

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In Vitro Micropropagation of Fruit Species Using Next Generation Bioreactors

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HIGHLIGHTS

- Use of bioreactors in fruit species.

Abstract

This review provides a summary of the most recent advancements in bioreactor systems, which have become more popular over the past few decades due to the outstanding qualities they offer for the creation of plant tissue and organ cultures in the laboratory as well as on a large scale. It provides a comprehensive discussion on the application of bioreactor systems in fruit cultivation as well as current research. This review presents a solution for researchers who are interested in the cultivation of diverse fruit species, as well as describes the methods that are used in the bioreactor system to propagate various fruit species.

Keywords: Bioreactor systems, In vitro, PLANTFORM, RITA, SETIS, TIS

1. Introduction

With the world's population growing at an alarming rate, so does the demand for food. Food production cannot adequately fulfill people's nutritional needs. As a result, it must produce more crops per unit of area. Plant biotechnology, which increases and improves productivity, also allows for the production of additional food raw materials from small-scale areas. Plants have been highly beneficial to mankind since the beginning of time. Plant biotechnology advances aim to improve and replace certain plant components such as carbohydrates, proteins, lipids, and vitamins over time.

In recent years, plant tissue culture methods have become increasingly important in plant reproduction, resource conservation, and secondary metabolite production. These strategies provide long-term solutions to a variety of difficulties in new and medicinal plant breeding and conservation biology (Yoshimatsu 2008; González-Rábade et al. 2012; Hussain et al. 2012; Chandana et al. 2018; Chand-ran et al. 2020). Micropropagation allows for the rapid and low-cost production of a large number of plants (Ozkaynak and Samancı 2005). In addition to the numerous benefits of micropropagation, there are some drawbacks. Labor expenditures account for 45-60% of production costs in micropropagation. Furthermore, hyperhydricity, which results in substantial losses, necessitates the employment of a large number of culture containers and

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semi-solid nutritional media (Rathore et al. 2004; Berthouly and Etienne, 2005). There are challenges with the disproportionate distribution of plant growth regulators in semi-solid and solid cultures, as well as in the nutritional medium, and varying sensitivity levels in cultured tissues depending on agar brand (Gupta and Prasad 2006).

One of the most expensive tissue culture components is agar. As a result, a variety of thickening components, such as plant-derived starches (Babbar and Jain 1998), resins (Babbar et al. 2005), and resins (Teng and Liu 1993), have been investigated as alternatives to agar (Bhattacharya et al. 1994; Prakash et al. 2004). Furthermore, resins are less attractive because they do not completely harden the medium. In reality, there has yet to be developed a commercially effective low-cost gelling agent. In vitro micropropagation has become an incredibly expensive and time-consuming method as a result of these factors. More success was achieved in this area with the use of liquid cultures in bioreactors to improve shoot propagation rates, growth, and quality while lowering process costs.

Bioreactor systems are classified as largely closed and regulated systems in a liquid environment that operates under aseptic circumstances and controlled settings (pH, temperature, ventilation). It gives benefits such as easy management of environmental conditions, high yield, and quality plant production thanks to bioreactor systems. It also protects against both abiotic and biotic stressors. There are simple and effectively built bioreactors for molecular agriculture and phytoregulation de-toxification of hazardous substances, which are utilized in the manufacture of aromatic and therapeutic metabolites (Shaposhnikov et al. 2009).

2. Bioreactor System and Types

The challenges in alternative propagation methods have been attempted to be eliminated as a remedy to the problems faced in propagation with plant tissue culture techniques and conventional methods (Cheong 2012). Although biotechnological technologies can produce solutions to difficulties faced by traditional methods, they are expensive. In terms of cost, gelling agents rank first (Quiala et al. 2012). However, the sole objective of bioreactor systems is not to be an alternative, but to rapidly-produce a huge number of plants while lowering costs. Recently, temporary immersion bioreactor systems have been employed for this, which have numerous advantages over gelling agent-based approaches.

The bioreactor system technology has emerged with the in vitro micropropagation of *Begonia x hiemalis* in MS medium and erlenmeyer flasks with kinetin hormone. For propagation in these erlenmeyers, the plants were shaken at 180 rpm (Takayama and Misawa, 1981). Haris and Mason conducted the first investigation using this technique in 1983, using *Solanum tuberosum* and *Coffee arabicana* species (Harris and Mason, 1983; Etienne and Berthouly, 2002). This approach, which was first utilized in the 1990s, is more efficient than typical agar-containing micropropagation systems. (Alvard et al. 1993; Escalona et al. 1999). Teisson and Alvard (1995) developed bioreactor systems that combine the benefits of semi-solid and liquid culture. Bioreactor systems have therefore been employed for large-scale micropropagation of several plant species or plant parts (Paek et al. 2001). To prevent this issue, bioreactors with temporary immersion systems (TIS) boost the benefits of liquid cultures by ensuring explant *in vitro* performance (Godoy et al. 2017). TIS bioreactors, culture medium, and in vitro explants are immersed for short periods of time, just long enough for the plants to absorb nutrients and plant growth regulators. Aeration within the explant tank also leads to a high-pressure ambient gas exchange to the explants, resulting in the improved shoot or plant growth and development. Bioreactors offer numerous benefits and drawbacks (Table 1) (Georgiev et al. 2014).

Table 1. The general characteristics, benefits, and drawbacks of the most prevalent TIS

TIS	POWER INPUT	CONSTRUCTION MATERIALS	STERILIZATION	PROS AND COS
Twin-Flask	Pneumatic	Glass	Autoclavable	Complex automation Have a low moisture content in the headspace The nutrient medium cannot be Replenished
RITA	Pneumatic and gravity	Polypropylene	Autoclavable	It has a basic automation system. Simple to use and safe functioning system Have a high level of humidity in the headspace With limited device positioning space
SETIS	Pneumatic and gravity	Polypropylene	Autoclavable Gamma irradiation	With basic automation that is simple to utilize It has a big region that is lit Enhanced drainage system Low energy consumption Low initial investment
PLANTFORM	Pneumatic and gravity	Polycarbonate	Autoclavable	There is no nutritional medium replenishment Reliable operation Easy to handle Easy access to light The apparatus is stacked on top of each other
PLANTIMA	Pneumatic and gravity	Polycarbonate	Autoclavable	No nutrient media replenishment Reliable operation Simple automation The apparatus is stacked on top of each other Low investment cost No nutrient media replenishment

TIS bioreactor systems are commonly used in horticultural crop research. This technique is defined as a method of developing physiologically healthy plants while decreasing plant hyperhydricity. TIS is a system in which plant cells, tissues, and organs are semi-automatically immersed in a liquid media for a set amount of time in a bioreactor (Hwang et al. 2022). By enhancing the airflow of the culture container, this device boosts the development rate of plants in many species (Bello-Bello et al. 2019). TIS promotes physiological processes such as photosynthesis, respiration, chlorophyll formation, and stomatal function during acclimatization, allowing plants to adapt well to the ex vitro environment (Aragón et al. 2014).

Transient Immersion (RITA®), Transient Immersion Bioreactor (TIB®), and Tidal Bioreactor (Tisserat and Vandercook, 1985; Ducos et al. 2007) are the most common TIS (Figure 1). MATIS® (Etienne et al., 2013) and SETIS (Hwang et al. 2022) are both Monoblock Forward Temporary Immersion Systems (Figure 2). The goal of this study was to find the best medium for *Schisandra chinensis* plants in two temporary immersion bioreactor systems, RITA® (Figure 4) and PlantForm bioreactor system (Figure 3). The tests lasted between 20 and 60 days. As a consequence, of the evaluated bioreactors, the RITA® bioreactor produced the greatest results in terms of biomass production and lignan (a valuable substance that can help prevent chronic diseases such as some types of cancer and cardiovascular disease) (Rodrguez-Garca et al. 2019; Szopa et al. 2017).

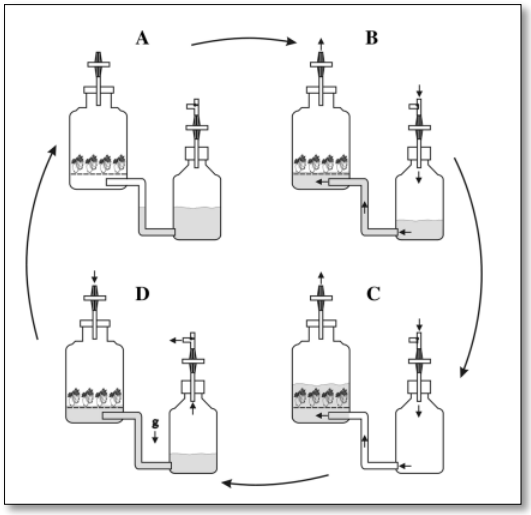

Figure 1. Technological design and operating principle of the Twin-Flask bioreactor system: (A) exposure time (B) Displacement of the liquid medium. Air pressure is applied to the media storage tank and the liquid media moves into the culture chamber; (C) immersion time; (D) evacuation of the nutrient medium. The air pressure is turned off and the medium flows back into the middle storage tank due to gravity.

SETIS is a TIS bioreactor with the advantage of a relatively large culture tank, approximately 6 L, and ease of operation (Kim et al. 2020). It has successfully produced virus-free apple seedlings (*Malus domestica*) in TIS, and sweet cherry (*Prunus ovatum*) and *Colocasia esculenta* seedling growth has also been reported in this system (Godoy et al. 2017; Arano-Avalos et al. 2020). Temporary immersion systems (TIS) are successful based on immersion period, frequency, and fluid volume per explant (Martnez-Estrada et al. 2019). With the broad adoption of these systems in recent years, it has become one of the most widely utilized micropropagation systems (Ruta et al. 2020). TIS is also known to have a positive effect on physiological processes such as stomatal function and chlorophyll production.

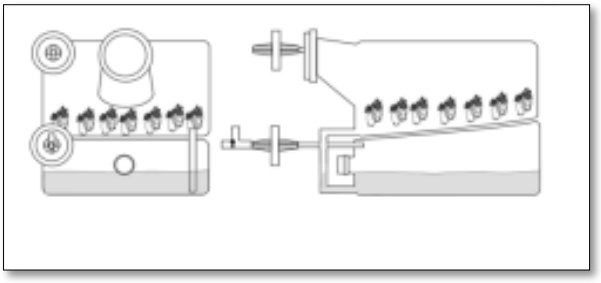

Figure 2. SETIS bioreactor systems

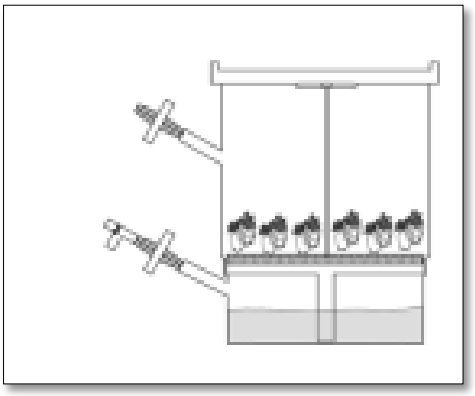

Figure 3. PLANKIMA bioreactor system

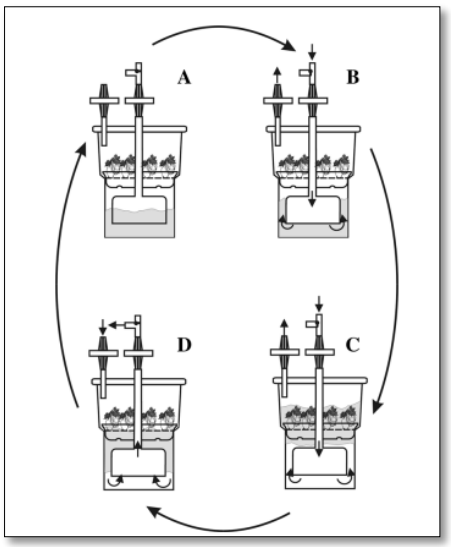

Figure 4. Technological design and operating principle of the RITA system: (A) exposure time; (B) Displacement of the liquid medium. Air pressure is applied to the lower chamber through the middle pipe. Liquid media moves to the upper chamber; (C) immersion time; (D) evacuation of the nutrient medium. The airflow is stopped and the medium flows back into the lower chamber due to gravity.

2.1 Studies in Fruit Growing

The TIS system was employed for the first time in banana micropropagation (Alvard et al. 1993). Many investigations using TIS have been reported to overcome the constraints of semisolid and liquid culture (Businge et al. 2017; Posada-Pérez et al. 2017).

Attempting propagation with a bioreactor system can yield successful results, especially in species that could not achieve successful outcomes with micropropagation (Hautsalo et al. 2017). Cuttings are used to propagate blackcurrant (*Ribes rubrum* L.), which is not an appropriate method. Their goal in their work is to establish an appropriate micropropagation methodology for blackcurrant and to investigate the impacts of various LED lighting. Combinations of white, red, and blue wavelengths improved the shot's quality. They also discovered that using a bioreactor boosted plantlet shoot height and roots. However, a multitude of procedures for blackcurrant propagation, roots, and acclimation are now available for use.

PlantForm and conventional tissue culture procedures were used to perform micropropagation and rooting studies on five different blackberry cultivars (Black Diamond, Black Pearl, Chester, Triple Crown, and New Berry). Plant growth regulators BA (Benzyl Adenine) and GA_3 (Gibberellic Acid) were added to the MS basic nutritional medium in solid culture micropropagation tests on five distinct blackberry cultivars. NAA (Naphthalene Acetic Acid) and IBA (Indole Butyric Acid) growth regulators were evaluated in MS nutritional medium for solid culture rooting studies. High micropropagation values were achieved from nutrient media containing 1 mg/L BA, and the best rooting success was obtained from 1 mg/L NAA plant growth regulator concentrations in the solid culture experiment findings. According to their findings, the PlantForm technology outperformed the solid culture approach for all five blackberry varieties. The PlantForm bio-reactor system was shown to be more effective than solid culture media for micropropagation and roots in this study (Umarusman et al. 2020).

They wanted to test a novel type of TIS bioreactor with *Myrtus communis* L. agar medium, a therapeutic aromatic plant species. For propagation, they employed MS medium supplemented with 1 mg/l BAP and 1 mg/l IBA. The solid agar medium and two different immersion periods (4 and 8 hours) were compared. After 8 hours of immersion, they got improved results. Plantlets cultivated in PlantForm bioreactor systems outperformed those grown in solid media. They discovered that their outcomes in micropropagation and rooting were once again more successful (Aka Kaçar et al. 2020

It has been attempted to reproduce in high antioxidant content blueberry semi-solid and RITA® bioreactor culture dishes. It was grown from blueberry leaves in semi-solid DM and TDZ-containing medium. They got shoot elongation in the propagated shoots' agar-free and bioreactor systems (Debnath 2011).

In raspberry and strawberry plants, TIS and RI-TA® type bioreactors were compared. Fresh weights rose in both types of TIS bioreactors. This rate rose dramatically in liquid culture compared to solid culture in strawberry plants in bioreactor systems. Micropropagation for strawberries can be done entirely in a liquid medium, while for raspberries, there is a risk of hyperhydration if the plants are kept in a liquid medium for too long. A protocol for mass propagation of raspberries is given, which combines plant propagation in a liquid medium (TIS bioreactor) and roots in a solid medium (Georgieva et al. 2016).

The TIS bioreactor system was used to study the micropropagation of axenic shoots generated from the germination of *Pistacia lentiscus* L. seeds. Different immersion times (5, 10, 15 minutes) and frequencies (4, 8, 16, 24, 32 hours) investigations were attempted. The best immersion frequency and time were determined to be 32 hours and 10 minutes. They observed that the vitrification rate in TIS trials fell from 100% to 8% as a result of changes in immersion frequency and time. *Pistacia* species can benefit from bioreactors. Studies have also revealed that it may be suitable as a medium for protoplast culture (Ekingen 2016).

The development of *Myrtus communis* L. and *Olea europaea* was compared in semi-culture and PlantForm bioreactor systems. They concluded that PlantForm bioreactor systems improved plant survival and quality in both species. During the reproductive phase of olives, the zeatin hormone offers a favorable reproduction environment. This system increases the rate of growth at lower concentrations. As a result, the bioreactor system produced superior outcomes (Carla et al. 2015).

Dipping was used for 2 minutes every 4 hours in a semi-solid nutrition medium and bioreactor system in a study on the banana plant, an industrial fruit crop in high demand in export markets. They were evaluated at two TDZ doses (0, 0.125, and 0.250 mg/l). As a consequence, the temporary bioreactor system produced the best shoot propagation results at 0.125 mg/l TDZ concentration (Daungban et al 2017).

3. Conclusions

The introduction of bioreactor systems and their usage in in vitro plant cells, as well as current fruit investigations, were assessed in this review. The usage of bioreactor systems in fruit cultivation, which began with the first banana (Alvard et al. 1993), has risen significantly in recent years among other species. Bioreactor systems, as opposed to the solid gelling agents used in *in vitro* micropropagation, are less expensive and produce a greater number of plants. Plant tissues that are constantly in contact with the nutritional media experience physiological concerns such as hyperhydration (vitrification). Because they make brief contact with the liquid nutritional media, temporary immersion bio-reactor systems reduce hyperhydration in plant tissues (Niemenak et al. 2008).

Many types of bananas (Alvard et al. 1993), raspberry and strawberry (Georgiev et al. 2014), olive (Carla et al. 2015), blackcurrant (Hautsalo et al. 2017), citrus rootstocks in the world and our country (Cengiz and Kaçar 2019), apple and sweet cherry seedlings production (Godoy et al. 2017; Arano-Avalos et al. 2020) and blackberry (Umarusman et al. 2020) bioreactor systems have been used successfully.

The Monoblock Forward Temporary Immersion System (MATIS® (Etienne et al. 2013)) and SETIS (Hwang et al. 2022) systems for *in vitro* micropropagation are being evaluated in comparison to more traditional methods such as semi-solid culture and liquid fixed cultures. In bioreactor systems, bulk propagation is more effective. Cost and labor savings are made, particularly in large-scale production. Studies have demonstrated that fruit species plants regenerate, develop, and accumulate biomass in bioreactor systems at their best rates. It demonstrated that an increase in cell size is the cause of the superb growth of regenerated plants cultivated in bioreactor systems. *Chrysanthemum* has demonstrated that secondary xylem formation from the stem is necessary for plant survival and growth following ex vitro transplantation (Hwang et al. 2022). The findings of this review demonstrated that bioreactor systems can be the best method for the industrial-scale production of fruit species, ornamental plants, and medicinal plants.

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References

Aka Kaçar Y, Biçen B, Şimşek Ö, Dönmez D, Erol M (2020). Evaluation and comparison of a new type of temporary immersion system (TIS) bioreactors for myrtle (*Myrtus communis* L.). *Applied Ecology and Environmental Research* 18 (1).

Akdemir H, Süzerer V, Onay A, Tilkat E, Ersali Y, Çiftçi YO (2014).Micropropagation of the pistachio and its rootstocks by temporary immersion system. *Plant Cell, Tissue and Organ Culture (PCTOC)* 117 (1): 65-76.

Alvard D, Cote F, Teisson C (1993). Comparison of methods of liquid medium culture for banana micropropagation. *Plant Cell, Tissue and Organ Culture* 32 (1): 55-60.

Aragón C, Sánchez C, Gonzalez-Olmedo J, Escalona M, Carvalho L, Amâncio, S (2014). Comparison of plantain plantlets propagated in temporary immersion bioreactors and gelled medium during *in vitro* growth and acclimatization. *Biologia Plantarum* 58 (1): 29-38.

Arano-Avalos S, Gómez-Merino F C, Mancilla-Álvarez E, Sánchez-Páez R, Bello-Bello JJ (2020) .An efficient protocol for commercial micropropagation of malanga (*Colocasia esculenta* L. Schott) using temporary immersion. *Scientia Horticulturae* 261: 108998.

Babbar S, Jain N (1998). Isubgol'as an alternative gelling agent in plant tissue culture media. *Plant Cell Reports* 17 (4): 318-322.

Babbar S, Jain R, Walia N (2005). Guar gum as a gelling agent for plant tissue culture media. *In Vitro Cellular & Developmental Biology-Plant* 41 (3): 258-261.

Bello-Bello JJ, Cruz-Cruz CA, Pérez-Guerra JC (2019). A new temporary immersion system for commercial micropropagation of banana (Musa AAA cv. Grand Naine). *In Vitro Cellular & Developmental Biology-Plant* 55 (3): 313-320.

Berthouly M, Etienne H (2005). Temporary immersion system: a new concept for use liquid medium in mass propagation, In: Liquid culture systems for in vitro plant propagation, Eds: Springer, p. 165-195.

Bhattacharya P, Dey S, Bhattacharyya BC (1994). Use of low-cost gelling agents and support matrices for industrial scale plant tissue culture. *Plant Cell, Tissue and Organ Culture*, 37 (1): 15-23.

Businge E, Trifonova A, Schneider C, Rödel P, Egertsdotter U (2017). Evaluation of a new temporary immersion bioreactor system for micropropagation of cultivars of eucalyptus, birch and fir. *Forests* 8 (6): 196.

Carla B, Fernanda C, De Carlo A (2015). Plant Form', a temporary immersion system, for in vitro propagation of Myrtus communis and Olea europaea, *The 6th International ISHS Symposium on Production and Establishment of Micropropagated Plants (PEMP) Sanremo Italy*, 19-24.

Cengiz M, Kaçar YA (2019). Micropropagation of some citrus rootstocks with classical and new generation tissue culture techniques. *Turkish Journal of Agriculture-Food Science and Technology* 7 (9): 1469-1478.

Chandana B, Kumari Nagaveni H, Lakshmana D, Shashikala SK, Heena M (2018). Role of plant tissue culture in micropropagation, secondary metabolites production and conservation of some endangered medicinal crops. *J. Pharmacogn. Phytochem* 3: 246-251.

Chandran H, Meena M, Barupal T, Sharma K (2020). Plant tissue culture as a perpetual source for production of industrially important bioactive compounds. *Biotechnology Reports* 26: e00450.

Cheong EJ (2012). Biotechnological approaches for improvement and conservation of Prunus species. *Plant biotechnology reports* 6 (1): 17-28.

Daungban S, Pumisutapon P, Topoonyanont N, Poonnoy P (2017). Effects of explants division by cutting, concentrations of TDZ and number of sub-culture cycles on propagation of 'Kluai Hom Thong'banana in a temporary immersion bioreactor system. *Thai Journal of Science and Technology* 6 (1): 89-99

Debnath SC (2011). Adventitious shoot regeneration in a bioreactor system and EST-PCR based clonal fidelity in lowbush blueberry (*Vaccinium angustifolium* Ait.). *Scientia Horticulturae* 128 (2): 124-130.

Ducos J, Labbe G, Lambot C, Pétiard V (2007). Pilot scale process for the production of pre-germinated somatic embryos of selected robusta (*Coffea canephora*) clones. *In Vitro Cellular & Developmental Biology-Plant* 43 (6): 652-659.

Ekingen Z (2016). Pistacia lentiscus L. tohumlarının çimlendirilmesi ile elde edilen aksenik sürgünlerin TIS biyoreaktör sistemi ile mikroçoğaltılması/Micropropagation of axenic shoots derived from germinated *Pistacia lentiscus* L. seeds using TIS bioreactor system.

Escalona M, Lorenzo J, González B, Daquinta M, González J, Desjardins Y, Borroto C (1999). Pineapple (*Ananas comosus* L. Merr) micropropagation in temporary immersion systems. *Plant Cell Reports* 18 (9): 743-748.

Etienne H, Berthouly M (2002). Temporary immersion systems in plant micropropagation. *Plant Cell, Tissue and Organ Culture* 69 (3): 215-231.

Etienne H, Bertrand B, Georget F, Lartaud M, Montes F, Decham E, Verdeil JL, Barry-Etienne D (2013). Development of coffee somatic and zygotic embryos to plants differs in the morphological, histochemical and hydration aspects. *Tree Physiology* 33 (6): 640-653.

Georgiev V, Schumann A, Pavlov A, Bley T (2014). Temporary immersion systems in plant biotechnology. *Engineering in life sciences* 14 (6): 607-621.

Georgieva L, Tsvetkov I, Georgieva M, Kondakova V (2016). New protocol for in vitro propagation of berry plants by TIS bioreactor. *Bulgarian Journal of Agricultural Science* 22 (5): 745-751.

Godoy S, Tapia E, Seit P, Andrade D, Sánchez E, Andrade P, Almeida AM, Prieto H (2017). Temporary immersion systems for the mass propagation of sweet cherry cultivars and cherry rootstocks: development of a micropropagation procedure and effect of culture conditions on plant quality. *In Vitro Cellular & Developmental Biology-Plant* 53 (5): 494-504.

González-Rábade N, del Carmen Oliver-Salvador M, Salgado-Manjarrez E, Badillo-Corona JA (2012). In vitro production of plant peroxidases—a review. *Applied biochemistry and biotechnology* 166 (7): 1644-1660.

Gupta SD, Prasad V (2006). Matrix-supported liquid culture systems for efficient micropropagation of floricultural plants. *Floriculture, ornamental and plant biotechnology: advances and topical issues* 2: 488-495.

Harrıs RE, Mason EB (1983). Two machines for in vitro propagation of plants in liquid media. *Canadian Journal of Plant Science* 63 (1): 311-316.

Hautsalo J, Rantala S, Rantanen M, Nukari A (2017). Culture medium, LEDs and bioreactor to improve in vitro propagation of red currant, *VII International Symposium on Production and Establishment of Micropropagated Plants 1224*, 209-216.

Hussain MS, Fareed S, Saba Ansari M, Rahman A, Ahmad IZ, Saeed M (2012). Current approaches toward production of secondary plant metabolites. *Journal of Pharmacy & Bioallied Sciences* 4 (1): 10.

Hwan HD, Kwon SH, Murthy HN ,Yun SW, Pyo SS, Park SY (2022). Temporary Immersion Bioreactor System as an Efficient Method for Mass Production of In Vitro Plants in Horticulture and Medicinal Plants. *Agronomy* 12 (2): 346.

Ivanova M, Van Staden J (2011). Influence of gelling agent and cytokinins on the control of hyperhydricity in Aloe polyphylla. *Plant Cell, Tissue and Organ Culture (PCTOC)* 104 (1): 13-21.

Kim NY. Hwang HD, Kim JH, Kwon BM, Kim D, Park SY (2020). Efficient production of virus-free apple plantlets using the temporary immersion bioreactor system. *Horticulture, Environment, and Biotechnology* 61 (4): 779-785.

Martínez-Estrada E, Islas-Luna B, Pérez-Sato JA, Bello-Bello JJ (2019). Temporary immersion improves in vitro multiplication and acclimatization of Anthurium andreanum Lind. *Scientia Horticulturae* 249: 185-191.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia plantarum* 15 (3): 473-497.

Niemenak N, Saare-Surminski K, Rohsius C, Ndoumou DO, Lieberei R (2008). Regeneration of somatic embryos in Theobroma cacao L. in temporary immersion bioreactor and analyses of free amino acids in different tissues. *Plant Cell Reports* 27 (4): 667-676.

Özkaynak E, Samancı B (2005). Mikroçoğaltımda Alıştırma. *Selcuk Journal of Agriculture and Food Sciences* 19 (36): 28-36.

Paek KY, Hahn EJ, Son SH (2001).Application of bioreactors for large-scale micropropagation systems of plants. *In Vitro Cellular & Developmental Biology-Plant* 37 (2): 149-157.

Posada-Pérez L, Montesinos YP, Guerra DG, Daniels D, Gómez-Kosky R (2017). Complete germination of papaya (*Carica papaya* L. cv.Maradol Roja) somatic embryos using temporary immersion system type RITA® and phloroglucinol in semi-solid culture medium. *In Vitro Cellular & Developmental Biology-Plant* 53 (5): 505-513.

Prakash S, Hoque M, Brinks T (2004). Culture media and containers, Low cost options for tissue culture technology in developing countries, FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, 29-4

Quiala E, Cañal MJ, Meijón M, Rodríguez R, Chávez M, Valledor L, de Feria M, Barbón R (2012). Morphological and physiological responses of proliferating shoots of teak to temporary immersion and BA treatments. *Plant Cell, Tissue and Organ Culture (PCTOC)* 109 (2): 223-234.

Rathore J, Rathore V, Shekhawat N, Singh R, Liler G, Phulwaria M, Dagla H (2004). Micropropagation of woody plants, In: Plant biotechnology and molecular markers, Eds: Springer, p. 195-205.

Rodríguez-García C, Sánchez-Quesada C, Toledo E, Delgado-Rodríguez M, Gaforio JJ (2019). Naturally lignan-rich foods: A dietary tool for health promotion? *Molecules* 24 (5): 917.

Ruta C, De Mastro G, Ancona S, Tagarelli A, De Cillis F, Benelli C, Lambardi M (2020). Large-scale plant production of Lycium barbarum L. by liquid culture in temporary immersion system and possible application to the synthesis of bioactive substance. *Plants* 9 (7): 844.

Shaposhnikov S, Frengen E,Collins AR (2009). Increasing the resolution of the comet assay using fluorescent in situ hybridization—a review. *Mutagenesis* 24 (5): 383-389.

Szopa A, Kokotkiewicz A, Luczkiewicz M, Ekiert H, (2017). Schisandra lignans production regulated by different bioreactor type. *Journal of biotechnology* 247: 11-17.

Takayama S, Misawa M (1981). Mass propagation of Begonia× hiemalis plantlets by shake culture. *Plant and Cell Physiology* 22 (3): 461-467.

Teisson C, Alvard D (1995). A new concept of plant in vitro cultivation liquid medium: temporary immersion, In: Current issues in plant molecular and cellular biology, Eds: Springer, p. 105-110.

Teisson C, Alvard D (1995). A new concept of plant in vitro cultivation liquid medium: temporary immersion, In: Current issues in plant molecular and cellular biology, Eds: Springer, p. 105-110.

Teng WL, Liu YJ (1993). Restricting hyperhydricity of lettuce using screen raft and water-absorbent resin. *Plant Cell, Tissue and Organ Culture* 34 (3): 311-314.

Tisserat B, Vandercook CE (1985). Development of an automated plant culture system. *Plant Cell, Tissue and Organ Culture* 5 (2): 107-117.

Umarusman MA, Şimşek Ö, Biçen B, Serçe S, Kaçar YA (2020). Farklı Böğürtlen (*Rubus fruticosus* L.) Çeşitlerinin Klasik ve Yeni Nesil Doku Kültürü Teknikleri ile Mikroçoğaltım Olanaklarının Araştırılması. *Alatarım* 19 (2): 75-84.

Yoshimatsu K (2008) Tissue culture of medicinal plants: micropropagation, transformation and production of useful secondary metabolites .*Studies in Natural Products Chemistry* 34: 647-752.