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Installation, Operation and Sustainability of the Temporary Immersion Bioreactor System

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Abstract The national biotechnology development agency acquired the technology for the Temporary Immersion Bioreactor System. This is an automated system for mass propagation of elite plantlets. Since its acquisition in 2010, two establishments, National Center for Genetic Resource and Biotechnology (NAGRAB) and Sheda Science and Technology Complex (SHESTCO) are currently using TIBS. Some of the plantlets that have been successfully propagated using TIBS include pineapple, mango, banana and sugarcane. The TIBS is being installed at some NABDA centers of excellence for the propagation of trees that would aid in the prevention of desert encroachment. NABDA in collaboration with Raw Materials Research and Development (RMRDC) is currently installing TIBS for the propagation of planting materials (Valencia Orange, Mango and Pineapple) for orchards needed for the Fruit Juice industry. Through our contacts with these installed TIBS, we have modified the assembly so that the immersion timings and electrical systems are fully automated and a backup system put in place in case of power failure which in consequence will produce a higher and more uniform yield. We also recommend the use of solar panels as another power backup option that can equally reduce cost.

Keywords TIBS (Temporary Immersion Bioreactor Systems), Sustainability, Installation, Cost, Micropropagation, Plantlets

Introduction

During the last thirty years, tissue culture-based plant propagation has emerged as one of the leading global agro-technologies. Between 1986 and 1993, the worldwide production of tissue cultured plants increased 50%. In 1993, the production was 663 million plants. By 1997, production had risen to 800 million plants. During 1990–1994, the micropropagation industry declined in Europe, mainly due to production shifting to developing countries, but since then because of the demand for high quality and quantity of plantlets, production in European countries has increased. Since 1995, production has increased by 14% in Asian countries, mainly due to the market entry of China, while the increase in South and Central America was from production in Cuba. More recently, some companies from Israel, the USA and UK have shifted their production requirements to Costa Rica and India. Tissue cultured plants have as yet to reach many growers and farmers in the developing countries. The primary advantage of micropropagation is the rapid production of high quality, disease-free and uniform planting material. The plants can be multiplied under a controlled environment anywhere, irrespective of the season and weather, on a year-round basis. Production of high quality and healthy planting materials of ornamentals, forest and fruit trees propagated from vegetative parts has created new opportunities in global trade for producers, farmers, nursery owners, and for rural employment.

Micropropagation technology however is more expensive than the conventional methods of plant propagation, and requires several types of skills. It is a capital-intensive industry, and in some cases the unit cost per plant becomes unaffordable. The major reasons are cost of production and know-how. During the early years of the technology, there were difficulties in selling tissue culture products because the conventional planting materials were much cheaper. Various attempts are being made to address this problem by inventing reliable and cost effective tissue culture methods without compromising on quality. This includes a constant monitoring of the input costs of chemicals, media, energy, labour and capital. In the industrialised countries, labour is the main factor that contributes to the high cost of production of tissue-cultured plants. To reduce such costs, some steps can be partially mechanized such as using peristaltic pump for medium dispensing, and of dishwashers for cleaning containers. In the less developed countries of Africa, Asia, and Latin America, where labour is relatively cheaper, consumables such as media, culture containers, and electricity make a comparatively greater contribution to production costs. For example, the cost of medium preparation (chemicals, energy and labour) can account for 30–35% of the micropropagated plant production.

In order to overcome the difficulties encountered in the use of plant tissue culture technology, bioreactors consisting of vessels have been designed for large scale cell, tissue or organ culture in media which can produce millions of elite plantlets (Table 1). The design for intermittent immersion of the plant tissue in the media is referred to as Temporary Immersion Bioreactor Systems (TIBS). The TIBS is designed based on temporarily immersing the tissue in nutrients and withdrawing at intervals. TIBS is a relatively recent micropropagation technique that employs the use of automated gadgets for rapid multiplication of plantlets under adequate conditions. It provides a more precise control of the adequate conditions (gaseous exchange, illumination etc.) required by plants for growth, development and survival than the conventional culture vessels. The bioreactor system incorporates a number of features specifically designed to simplify its operation and reduce production costs [1]. The System in addition is advantageous in the areas of having diminished production costs regarding labour force, energy savings and augmented micropropagation productivity and efficiency. Also, while using less area this system leads to a substantial increase in the production capabilities of biofactories. Automated micro propagation through bioreactors has greater feasibility of producing greater plantation volume and they have been designed using an automated system which provides maximum opportunities for monitoring and control of environmental conditions.

Table 1: Comparison of Yield between Conventional Micropropagation and Temporary Immersion Systems (TIBS) [2]

Crop	Variety	Conventional Micropropagation (Ton/Hec)	Temporary Immersion Systems (Ton/Hec)
Pineapple	Smooth cayenne	8.0	68.8
	MD2	5.8	26.8
Sugarcane	C91-301	3.7	34.1
	C1051-73	4.1	58.0
	C120-78	3.9	30.2
	C323-68	4.3	39.5
	Cp-5243	4.0	32.5
Taro	INIVIT	3.0	10.4
	Mexico 1	2.8	7.7
Banana	FHIA-18	3.8	7.4
	FHIA-01	3.4	10.4
	Grand Nane	4.0	16.6
Plantain	CEMSA ¾	2.5	7.8
Eucalyptus	Urograndis	2.7	11.6
Syngonium	W. Butterfly	7.3	28.0
	Pixle	2.2	18.4
Philodendron	Xanadu	2.0	8.8
Spathyphyllum	Sensation	3.7	17.6

These advantages notwithstanding, automated production processes based on pre-sterilized membrane capsules, bioreactors, mechanized explant transfer, and container sealing such as these are not commercially viable propositions in many developing countries. Therefore, low cost alternatives are needed to reduce production cost of tissue-cultured plants. Many of the low cost technology options can be incorporated in various steps of plant micropropagation. Low cost technology will be of great value for large scale multiplication of many economic trees and shrubs that are conventionally vegetatively propagated. In order to maximize the advantages derived from the use of bioreactors in tissue culture, the National Biotechnology Development Agency (NABDA) acquired the technology for rapid mass production of elite plantlets using TIBS. The technology was acquired from Technoazucar(now known as Azutecnia), the Sugar Company of Cuba. NABDA's first TIBS is currently domiciled at the National Center for Genetic Resources and Biotechnology (NACGRAB) in Ibadan, Oyo State. We have successfully installed another at our headquarters' laboratory Abuja with a modification to maximize its operational efficiency. While this paper is concerned with sustainability of the technology, it also addresses areas through which the efficiency can be increased and the overall cost further diminished to ensure delivery of plantlets at affordable costs in developing countries such as ours.

Mechanism of Operational Cycle

This system (Fig. 1) requires steady power supply for its successful operation. The air compressor is powered until the 25 liter capacity tank (as applied in this case study) is filled with air at a pressure of 8bar which could be regulated according to target requirement. The outlet pressure is set to 1.5bar taking into consideration important factors such as the air travelling distance, size and number of vessels in the system. The air is let into the system at required pressure and its flow is controlled by the solenoid valves. A total of four solenoid valves (1,2, 3 & 4) are needed to put the system in a functional state. The valves operate in pairs of inlet and outlet each with 1 & 4 being the inlet valves and 2 & 3 being the outlet valves respectively. For optimum operation, valve 1 pairs with valve 3 (inlet/outlet) and valve 4 pairs with valve 2. The operational cycles can be varied depending on the availability of power supply and plant nutrient requirements. The air is pneumatically driven through the connecting pipes via the solenoid valve allowing the flow of air into the system. This is achieved by the use of programmable timers which permit the flow of electric current at the designated periods. With this, pressurized air through the hydrophobic micropore filters is introduced into the bioreactor vessels, thus automating the system [1].

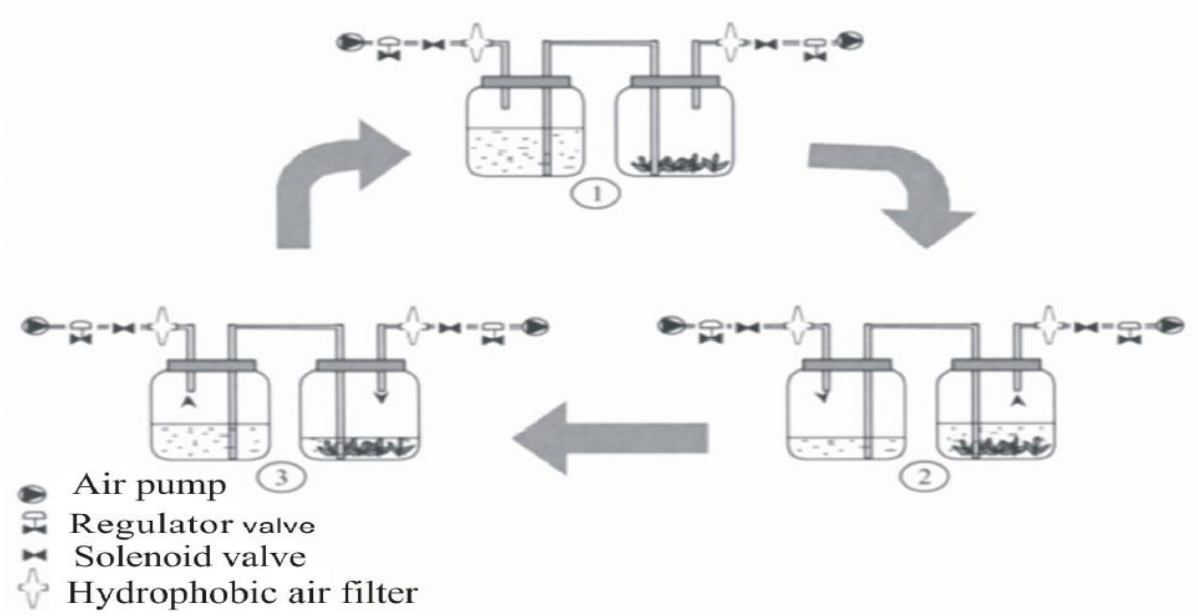

Figure 1: Schematic diagram of the set-up of the Temporary Immersion Bioreactor [3]

The system (Fig. 1) utilizes a pair of culture (Reaction) vessels, one containing the shoots and the second the liquid culture medium. Valve 3 (V3) is opened 10 sec prior to the opening of valve 1 (V1) to discharge excessive pressure in the system. This is to avoid damage or disconnection of connecting tubes and vessels resulting from high pressure. Pressurized air enters into bioreactor vessel A through the autoclavable tube 1 at the opening of valve 1. The air pressure in the vessel displaces the liquid medium through tube 2. The pumping duration of the medium is also dependent on the quantity of medium in the bottle and also the air pressure from the compressor. It is important to note that excessive bubbling during the feeding cycle has been reported by Ziv (2000) [4] to have a deterrent effect on plants in TIBS. The period of immersion could also vary, based on specific plant requirements. The name of the system is basically derived from this process where plants are immersed temporarily, and again the immersion period depends on individual plant species [1].

After completion of the desired immersion period, a reverse process similar to the process explained above takes places again, this time, returning media to vessel A. Valve 2 (outlet) is opened and valve 4 is also opened 10secs later. This is accompanied by the inflow of pressurized air via tube 3, displacing the medium in vessel B. The medium is withdrawn through tube 2 into vessel A. This cycle is continually repeated at the prescribed intervals (3 hours in this study) to ensure adequate nutrient uptake and nourishment of plants in TIBS.

Components of the Tibs And Their Functions

The Timers

The timer is an electronic device that regulates the inflow of compressed air and the duration of light. The timer used in our installation (Plate 1A) is the ENTES® (model MB-50). Timers are responsible for channeling signals to the various solenoids valves that allow for the passage of compressed air to the reaction vessels. We added an extra timer called the Primary Timer (Plate 1B) at the main power source thus ensuring that the system is fully automated. This primary timer regulates when the secondary timers, compressor and fluorescent light bulbs come on and go off.

The Contactors

Contactors (Plate 1A) are essentially the "bridge(s)" in this system. The main source of AC (alternating current) comes into these contactors and they further supply power to the timers and the fluorescent light bulbs. In an eventuality where there is a power surge, the timers do not get affected and the programs remain intact.

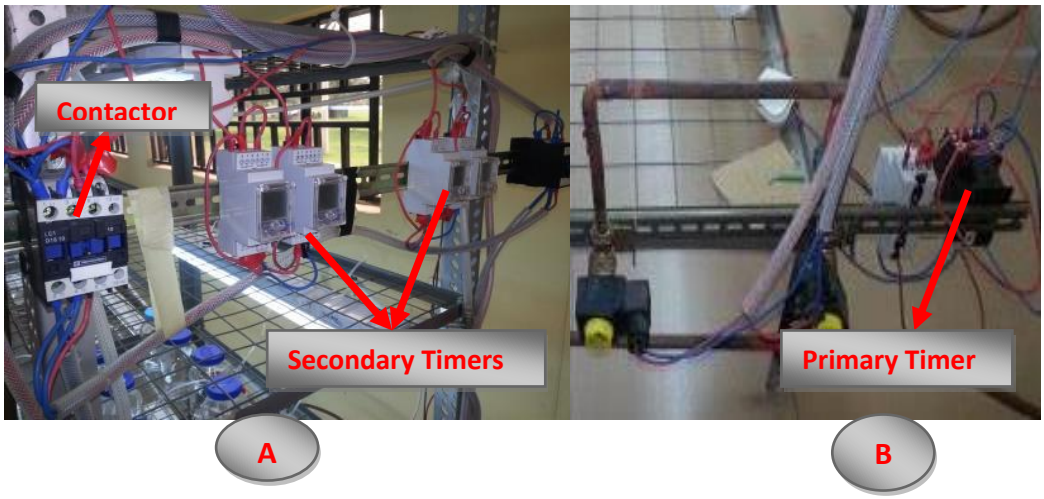

Plate 1A: A section of the TIBS showing Contactor and Secondary Timers

Plate 1B: A section of the TIBS showing the Primary Timer

The Solenoid Valves (Plate 2)

These are valves that regulate the inflow or outflow of air to or from the reaction vessels. Their activities are controlled by the timers and thus, they direct the air from the oil-free compressor to the respective reaction vessel.

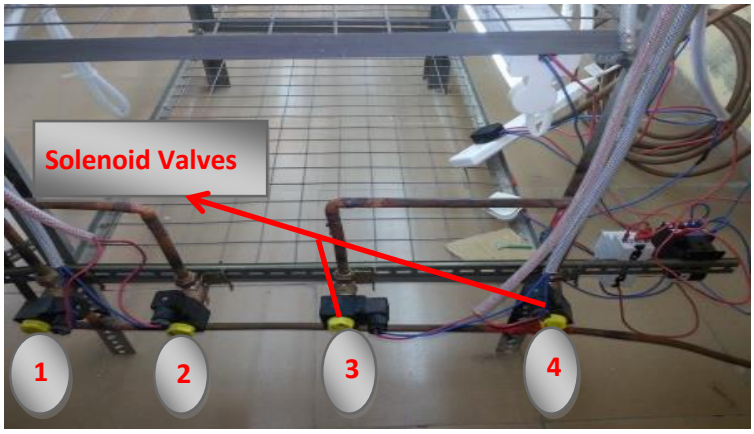

Plate 2: Arrangement of Solenoid Valves along the edge of the rack

The Fluorescent Light Bulbs

Fluorescent light bulbs are installed to mimic the natural sun light. White light is preferred because they generate little or no heat, therefore making it favorable for the setup in ensuring that the temperature desirable is maintained.

The Oil-Free Compressor

An oil free compressor (Plate 3) is preferred because it is electrically operated and thus making it possible for automation. Also there is no risk of introducing contaminants to the TIBS.

Plate 3: Oil- Free Compressor secured outside of the laboratory

The Hydrophobic Filters

22µm hydrophobic filters were installed in this case study (Plate 4). They are placed in between the air inlet and the reaction vessel. They serve to trap any microbial cell or particle from the pipes or tubes, allowing only air to reach the reaction vessel.

The Reaction Vessels

Borosilicate/Polycarbonate bottles which are paired up (Plates 4 & 5) are used because they are autoclavable ensuring that they can be reused and are kept sterile. In our installation, discarded bottles originally used in packaging salad dressings were found to be good substitutes for ready-made vessels. Various sizes can be used depending on the plant target number and also availability of materials.

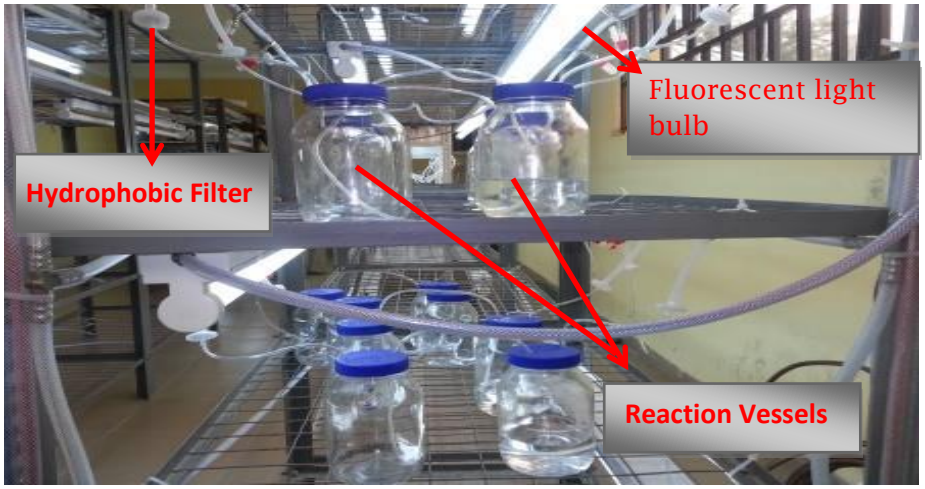

Plate 4: A section of Pairs of borosilicate/polycarbonate bottles and connected microfilters arranged on the shelves

Plate 5: Three rows of the bottles in pairs on the shelves

Power supply and Back-up

A power backup in form of a UPS was installed at the primary power supply point which ensures that in a situation where there is an outage, the back-up system supplies enough electricity for at least one more immersion.

Using Solar Panels as a Power Source for TIBS

We suggest the installation of solar panels as an alternative for conventional electricity. This would ensure that the problem of power failure is checked. The high cost of running generators would be eliminated. The initial installation cost might be expensive, but projected statistics shows that it is a lot cheaper than using conventional power supply and running back-up generators.

Current Uses of TIBS in Nigeria

In 2009, Raw Materials Research and Development Council (RMRDC) in collaboration with the fruit juice group of the Food, Beverage and Tobacco sector of Manufacturers Association of Nigeria (MAN) organized a

stake holders forum on "Sourcing of Raw materials for the Fruit Juice Industry" held at the MAN house, Ikeja, Lagos. NABDA at that meeting proposed the use of the use of Temporary Immersion Bioreactors for mass production of planting materials (Mango, Valencia orange and other fruit varieties).NABDA in collaboration with the RMRDC under the auspices of the Fruit Juice Initiative was funded through the World Bank Step-B grant to secure a TIBS for multiplication of elite plantlets of Fruit trees for the Fruit Juice Industry [5-6].

Since its introduction in Nigeria, Temporary Immersion Bioreactors have been used for mass production of elite plantlets of several economic plants. At the National Center for Genetic Resource and Biotechnology (NACGRAB), Ibadan, it has been used to produce Pineapple, Sugarcane, Banana and Eucalyptus plantlets most of which have been supplied to farmers.

Sheda Science and Technology Complex (SHESTCO) later acquired this technology from Technoazucar and has been able to mass produce a rescued variety of sugarcane for West African Sugar company.

Another installation waiting to be put into functional is at the University of Maiduguri(UNIMAID), the Borno State capital under the auspices of NABDA/UNIMAID STEP-B World Bank sponsored project. The purpose of this is to service the Afforestation programme aimed combating the menace of desert encroachment.

The TIBS when put to use in servicing the large volumes of plantlets required by various programmes such as the aforementioned and more, would generate appreciable funds that can partly sustain it while it is envisaged that the modifications made and suggested in our installation would equally contribute to its sustainability. NABDA, as a body responsible for the development and harnessing of biotechnology across the country has taken the lead in acquiring this technology for rapid mass production of planting materials in the country.

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