Mobile automated temporary immersion system for in *vitro* plant propagation

Sistema móvil de inmersión temporal automatizado de propagación *in vitro* de plantas

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Abstract

Introduction--- Colombia is currently transitioning to peace, and a key issue in this process is the restoration of land cultivation for affected farmers. Implementing systems and methods to automate micropropagation procedures could be a viable, low-cost option for producers.

Objective--- The goal is to support the in vitro propagation of plants using a mobile automated temporary immersion system device.

Methodology--- The device consists of a mobile structure for easy movement, glass containers, pneumatic lines, logic controllers, electrical connections, and a user interface.

Results--- The design and development of the proposed low-cost device enables the rapid, clean, and efficient propagation of crop planting materials.

Conclusions--- The developed device facilitates the mass propagation of selected plant materials in excellent health conditions, addressing the needs of producers for high-quality planting material.

Keywords--- Micropropagation; automated device; planting materials; producers; bioreactor.

Resumen

Introducción--- Colombia actualmente se encuentra en una transición hacia la paz y uno de los temas claves del posconflicto es la restitución de cultivos de tierras a los campesinos afectados. El uso de dispositivos y metodologías para automatizar los procedimientos de micropropagación puede ser una alternativa viable de bajo costo para los productores.

Objetivo--- Contribuir a la propagación in vitro de plantas por medio de un dispositivo móvil de inmersión temporal automatizado.

Metodología--- Se desarrolló un dispositivo con una estructura móvil para su fácil desplazamiento; recipientes de vidrio; línea neumática; controlador lógico; acometidas eléctricas e interfaz de usurario.

Resultados--- Se logró diseñar y elaborar un prototipo del dispositivo propuesto de bajo costo para la propagación rápida, limpia y eficiente de materiales de siembra de los cultivos.

Conclusiones--- El dispositivo desarrollado permitirá la propagación masiva de materiales vegetales seleccionados de siembra en excelentes condiciones de sanidad aportando a las necesidades de materiales de siembra de los productores.

Palabras clave--- Micropropagación; dispositivo automatizado; biorreactor; materiales de siembra; productores.

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I. INTRODUCTION

Colombia is currently undergoing a transition to peace, with a key aspect of this process being the restoration of land cultivation for affected farmers. [1] In this context, farmers stand to benefit significantly from the micropropagation of plant species, as it can substantially boost agricultural production in the affected areas [2]. However, traditional plant tissue culture methods for large-scale micropropagation remain economically unfeasible due to their high labour requirements and limited automation [3]-[4]. Conversely, micropropagation systems utilizing bioreactors based on the principle of temporary immersion systems (TIS) have proven to be more cost-effective [5]-[6]. Furthermore, these systems offer a more reliable approach for the successful production of in vitro seedlings, enhancing both the efficiency and scalability of agricultural propagation efforts.

In this regard, bioreactors present a viable option for large-scale seedling production compared to conventional micropropagation, offering several advantages. These include accelerated multiplication rates, increased productivity, uniformity in production, reduced labour requirements, and ultimately, a lower overall cost per unit [6]. Additionally, bioreactors enable temporary contact between cultures and the liquid medium, promoting normal plant growth while mitigating the issue of hyperhydricity.

There are several types of temporary immersion system (TIS) bioreactors, including the Receiver Automated Temporary Immersion System (RITA®) and the Twin Flasks System (BIT®). The RITA® system, first introduced by Vitropic in France [7], has been widely employed in numerous studies since its inception [8]. Similarly, the BIT® system, as described by [9], has also found extensive application in various research efforts [10]. The most recent advancement in TIS technology is the Plant Form system, introduced in 2014. This system was developed and is currently commercialized by Professor Margareta Welander from the Swedish University of Agricultural Sciences (SLU), Department of Plant Breeding [4].

However, these bioreactors are expensive for Colombian producers and difficult to handle due to their weight and small interior base, which often leads to disturbances in the culture explants [4]-[11]. Therefore, the objective of this research was to design and develop a low-cost, mobile automated temporary immersion system that is easy to transport, allowing for the rapid, clean, and efficient propagation of planting materials for priority crops in various regions of the country.

II. MATERIALS AND METHODS

The physical structure of the device, as informed by previous studies [12]-[13] (fig. 1) consists of the following:

1. A mobile structure to move the machine according to the user's needs, to the appropriate space or environment to carry out the process, facilitating supervision tasks, reconfiguration of cycles and activities related to the process that allow correct micropropagation.
2. The general structure, which is responsible for supporting the main instruments of the process, allows the proper location of each component that makes up the process.
3. The glass containers to simulate the artificial environment where the micropropagation of plants takes place.
4. The pneumatic line for air access and distribution, to apply the SIT micropropagation technique by bioreactor.
5. The programmable logic controller responsible for the automatic control of the process allows the reconfiguration of the times of each cycle of the micropropagation process. This enables the user to modify the process according to their needs.
6. The electrical connection for the control of each actuator (electro valve), the lighting system for process control and the electrical power supply.
7. The user interface through which the instructions given by the user are linked with the programmable logic controller and the process actuator.

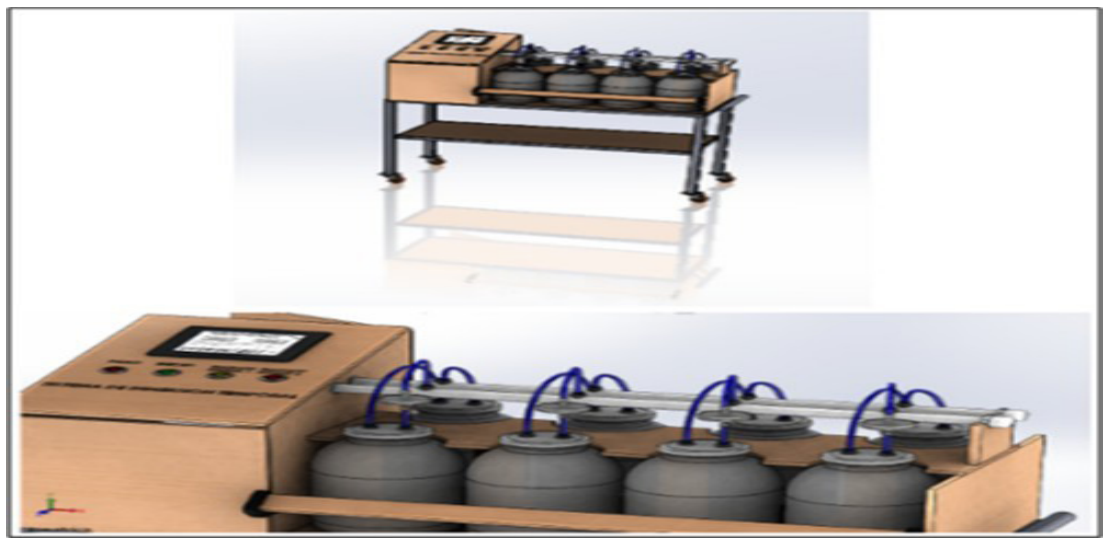


Fig. 1. CAD design of the device

Source: Authors

Mobile structure

The mobile structure is made with a 1-inch steel angular profile and ⅛" gauge, as can be seen in fig. 2. Likewise, it has a one-inch square steel tube that supports the four corners of the structure, they are joined using 3/32-gauge 6013 welding. It is also covered with a layer of anticorrosive paint to protect it from factors such as humidity and corrosion, after which a layer of oil paint is applied for the final finish. It has 4 rolling bases, each capable of supporting a load with a total weight of 60 to 100 kilograms, allowing the movement of the device to different places. Furthermore, it has a predetermined design to fit into the overall structure and uses the lower space for compressor storage, reducing the amount of space required to store the device.

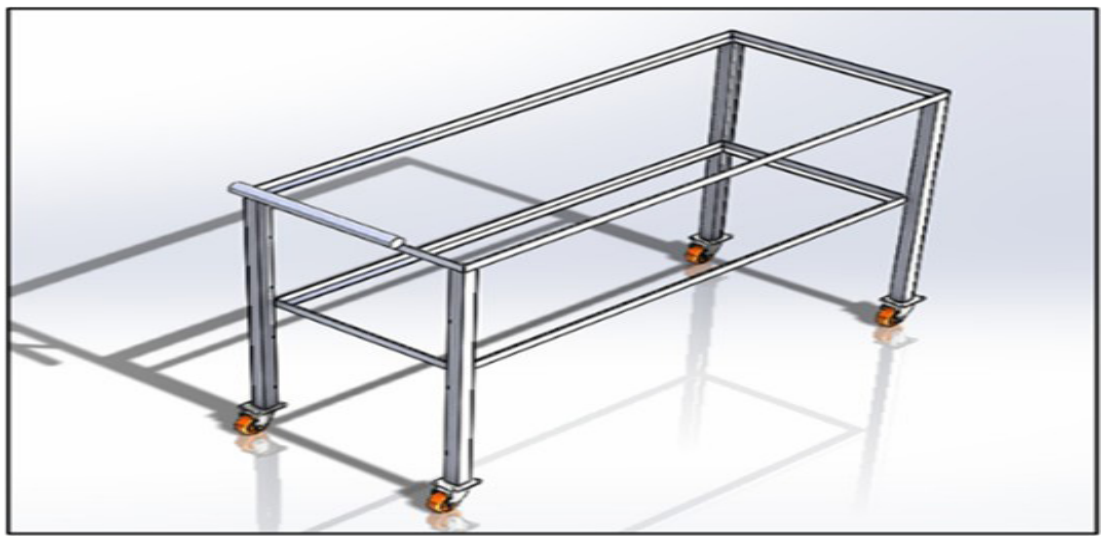


Fig. 2. CAD design of the mobile structure.

Source: Author(s)

General Structure

It is made with sheets of 9mm medium density fibreboard, or MDF, joined by quick assembly screws. The structure has a lacquer coating to protect each of the sheets, in fig. 3 you can see the structure composed of 10 parts.

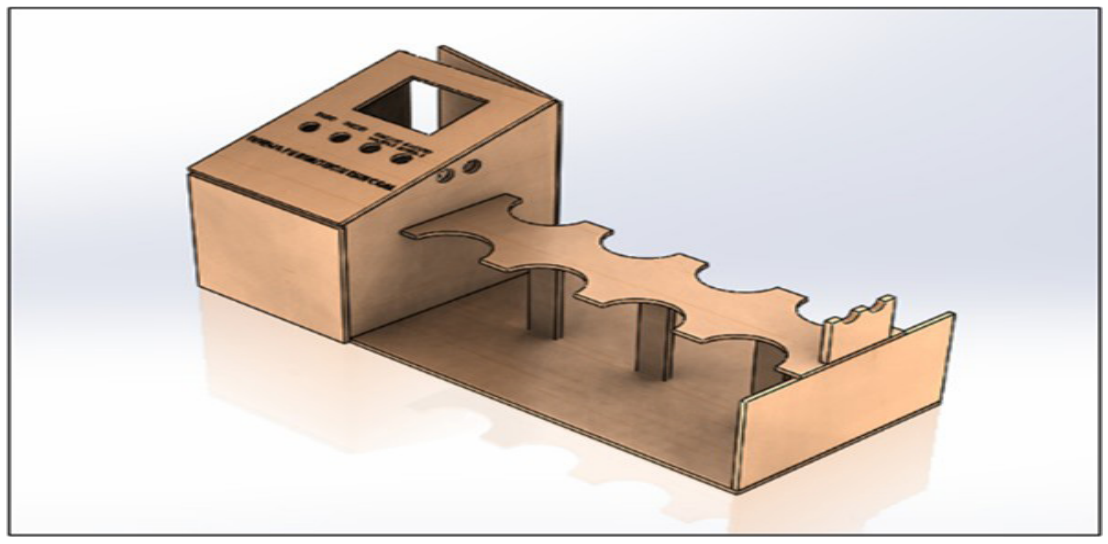
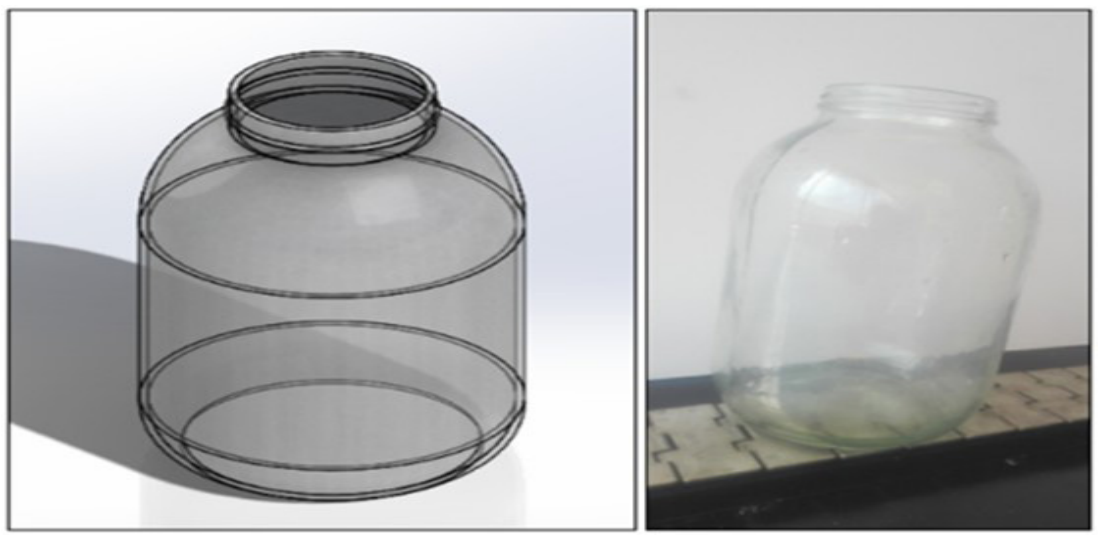


Fig. 3 Device general structure

Source: Author(s)

Glass containers

There are a total of eight glass containers to simulate the artificial environment where the micropropagation of each explant will take place. This container is called a bioreactor, which can be seen in fig. 4a-4b. Each one has a volume of 2600 cm3 and are located vertically.



a) CAD design b) Real bioreactor

Fig. 4. Glass container

Source: Author(s)

The lid, as shown in fig. 5, has two holes to adapt the pneumatic quick coupling fittings to the pneumatic line, allowing air to enter and the nutrient solution to exit towards the explant. The return of the nutritive solution is done by entering air into the bioreactor where the explant is located, and due to a pressure difference, it re-enters the original bioreactor.

Fig. 5. Glass container lid

Source: Author(s)

Pneumatic line

For the access and distribution of air in the temporary immersion bioreactor for the micro-propagation of plant species, the pneumatic line is made up of a compressor, solenoid valves, silicone hoses, quick pneumatic couplings, fittings, and distribution tubes to the containers.

Compressed air source

It is the air source that feeds the pneumatic circuit in charge of carrying out the movement of the nutritive culture medium. In the device, a small compressor is used that satisfies the required pressure of 20 PSI.

Solenoid valves

Two Festo brand solenoid valves shown in fig. 6 are used, electrically actuated by supplying 12 volts of direct current, which is controlled by the NAIS FP0-C10RS PLC, and the voltage supplied by a power source that converts alternating current from the commercial power line to direct current.

Fig. 6. Solenoid valves 12V DC

Source: Author(s)

Pressure regulating valve

This valve is responsible for maintaining the pressure at a non-dangerous limit for the optimal operation of the machine. It ensures that the amount of pressure in the containers is not too high. It is manually calibrated at a certain pressure, and when the pressure in the pneumatic circuit exceeds this point, the valve activates and lets out the excess air. When this pressure is below the established point, the valve automatically closes.

Air filter

It is a small filter located in the silicone hose that goes from the mother tube to the container, so that the air that enters the containers is in aseptic conditions, as shown in fig. 7, and it is of the utmost importance so that the explants do not contaminate.

Fig. 7. Air filter for connection with 6mm hoses

Source: Authors(s)

Silicone hoses (polyurethane)

At one end, 6mm silicone hoses are installed that fit each of the bioreactor lids, and at the opposite end, they are attached to the tubes responsible for distributing the air to each of the bioreactors, as shown in fig. 8.

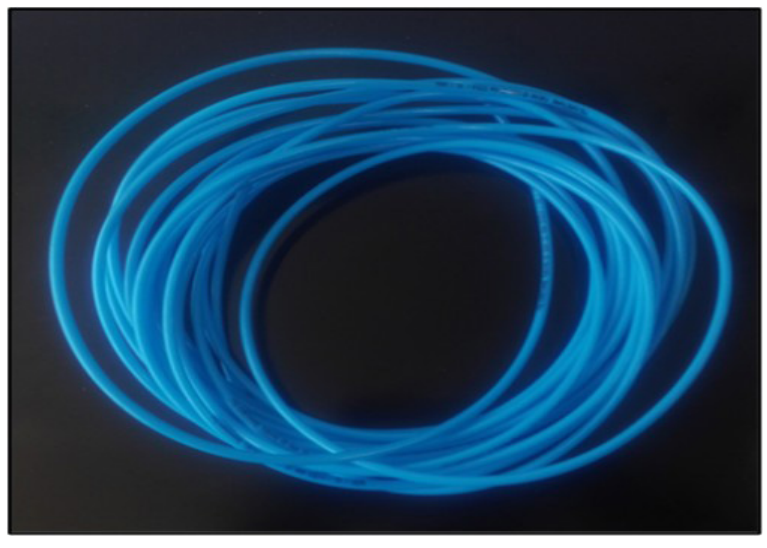


Fig. 8. Silicone hoses 6mm

Source: Author(s)

Pneumatic Quick Couplings (Fittings)

A total of 16 PC6-02 reference fittings are installed to join the hoses in charge of air mobilization. Each fitting has an external thread to fit the covers and move the air safely as can be seen in fig. 9.

Fig. 9. Pneumatic quick fitting coupling

Source: Author(s)

Distribution tubes

There are two half-inch diameter tubes responsible for distributing the air. As indicated in fig. 10, located horizontally, in which the containers of the explants and the nutrient solution or culture medium are connected.

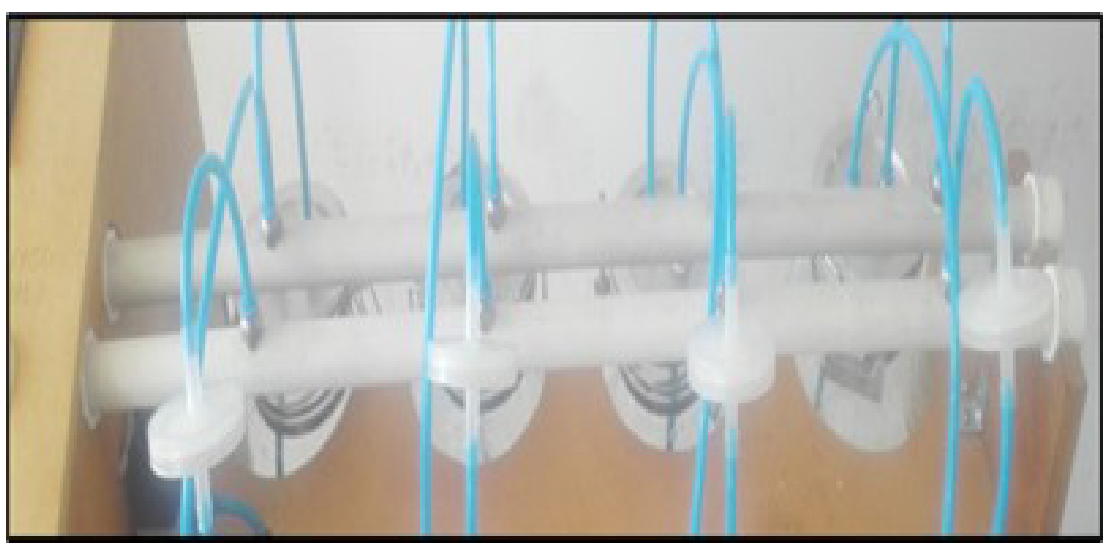


Fig. 10. Distribution mother tubes

Source: Author(s)

Programmable Logic Controller (PLC)

It is used for automatic process control, allowing the user to reconfigure the times of each cycle of the micropropagation process, giving the user advanced machine control as shown in fig. 11.

This device is responsible for governing each of the actions carried out by the plant: acti-

vation of solenoid valves, lighting control, activation of the compressor, communication with the HMI screen and reading of each digital signal found on the front panel: emergency stop, process start, manual transfer 1 and manual transfer 2.

The PLC used in this machine is a NAISS from MATSUSHITA-PANASONIC Reference FP0 - C10RS with the following characteristics:

✓ Input Specifications
Input voltage 24VCC
Voltage Margin 21,6 a 26,4 VCC
Input power 4,3 mA a 24 VCC
4 relay outputs
6 inputs

Removable screw terminals or MIL connector response time:

OFF-ON 50 us or less for X0, X1
OFF-ON 100 us or less for X2 to X5
OFF-ON 2 ms or less for X6 to XF
ON-OFF Equal

✓ Output Specifications
Contact type: Normally open
Switching capacity: 2A 250VAC, 2A 30VCC
Response time:
OFF-ON 10 ms or less
ON-OFF 8 ms or less

Fig. 11. PLC NAIS FP0 - C10RS
Source: Authors(s)

Electric system

In this section, all the systems and connections belonging to the electrical part will be referenced, both as the supply of electrical energy and as its distribution within the system.

Controller Power Supply

The electrical connection of the machine is made up of AWG 18 wiring; 24V DC is used to supply the power source to the PLC through an ATX source, from which 24V DC is derived for the digital signals that enter the PLC, such as emergency stop, start of process, manual transfer 1 and manual transfer 2. The machine uses low electrical power and due to this, the use of an ATX source is chosen. A gutter and shell are used for the correct distribution of the cabling. The fig. 12 shows the electrical diagram of the adopted controller, and fig. 13 the control panel from the inside that contains the electrical connections.

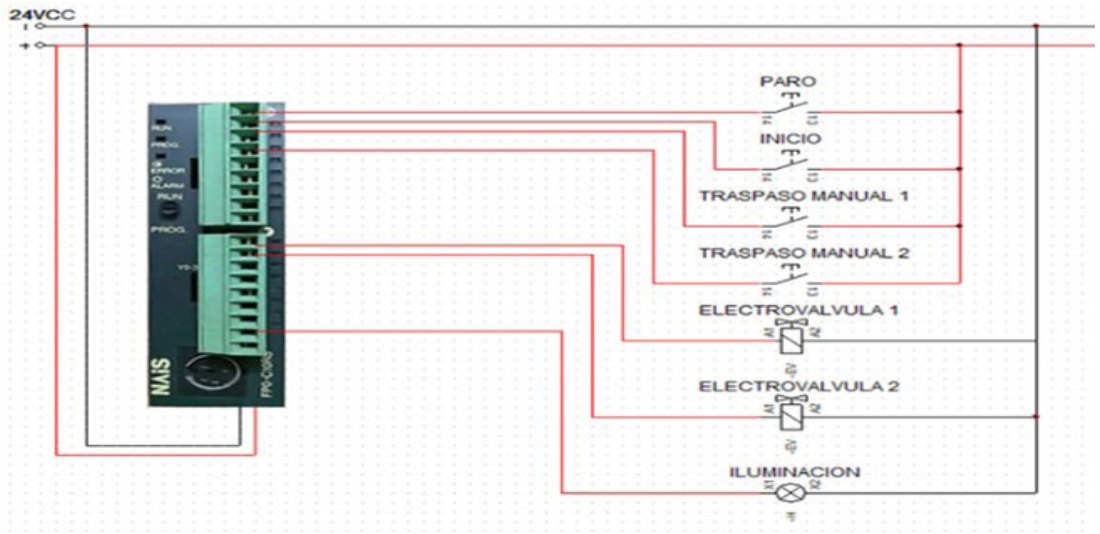


Fig. 12. Controller electrical connection

Source: Authors(s)

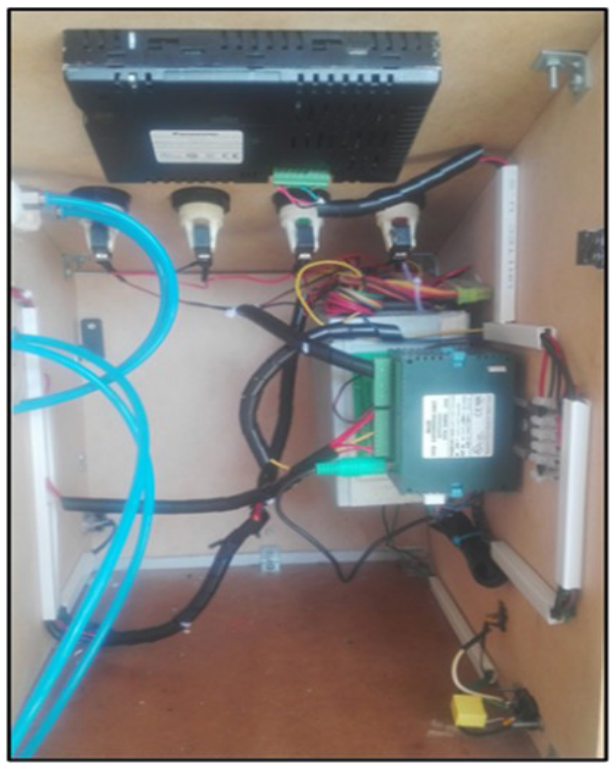


Fig. 13. Electrical connections

Source: Authors(s)

Lights

The lights, as shown in fig. 14, help develop the explants, and as such, the device can generally be in dark places. The explant container is in the middle of two light strips, one directly attached to the general structure and the other removable. The removable one has 2 supports that protrude from the front part of the structure. It also has a connector on the right side of the control panel.

Fig. 14. Light's location

Source: Author(s)

Electric Autonomy

The system has an input for the supply from the normal network and an auxiliary clean energy supply system independent of the electricity network. Likewise, it has a solar panel (fig. 15), that is, a device for inverting the electric wave, an MPPT controller (fig. 16), and a charge accumulator or battery that can be seen in fig. 17. The technical characteristics of each device are described below:

Solar Panel

Brand: POWEST
Model: CSG 150M2-36
Maximum Power: 150W
Open Circuit Voltage: 22.3V
Short Circuit Power: 8.51A
Voltage at Maximum Power: 18.7V

Fig. 15. Solar Panel

Source: Authors(s)

Sine wave inverter

Brand: Inti Photovoltaics
Model: IIP-12300
Continuous Power: 300VA
Peak Power: 600VA
Voltage DC:12V
Voltage AC:120/230V
Frequency: 50Hz/60Hz

MPPT Controller

Brand: Inti Photovoltaics
Model: ICM 1024 150
Battery entry voltage: 12V/24V
Rango de Voltage Photovoltaic: 18V-150V / 36V-120V
Max charge current: 10[a]

Fig. 16. Top MPPT controller bottom Inverter

Source: Authors

Charge accumulator
Brand: MARATHON
Voltage:12 V DC
Current: 155 Amp-hours
Connector: Screw Type Terminal
Weight: 53 kg

Fig. 17. Charge accumulator
Source: Authors(s)

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User interface

For the user interface, a Panasonic reference GT32M screen is implemented, shown in fig. 18. The technical characteristics of this HMI are:

Dimensions: 113,2 x 86,4 mm.
Display type: LCD, Monochrome
Resolution: 320 x 240 pixels
Operating voltage: 24VCC
Screen size: 5,7 inch
Backlight: LED White
Displayable Characters: 768
Memory Capacity: 12 Mbyte
External interface: USB port and slot for SD/SDHC.
Communication: RS232C

Fig. 18. Touch screen HMI display, Panasonic GT32M
Source: Author(s)

III. RESULTS

The applied process consisted of the micropropagation technique "Temporary immersion system by bioreactors" with the difference that the one used in the prototype was automated, which seeks to reduce human contact with the process, as shown in fig. 19.

Fig. 19. Final prototype

Source: Author(s)

It has four identical modules that work in parallel. The operation of this device must be supervised by a person with prior knowledge of biotechnology, specifically in plant tissue culture (micropropagation), in which identical plants are obtained from tissue from the mother plant (explant). This process is carried out in isolated containers where the explant will be periodically in contact with a liquid culture medium. To put this device into use, the explant is introduced into the indicated container, which must be previously sterilized.

The other glass container will contain a nutritious culture medium in which the explant from the primary container will be bathed at a time determined by the person in charge, which will vary depending on the species or variety. The time is entered through the HMI screen GT32M of the Panasonic brand that is in the control panel. The data is entered into a NAIS FP0C10-RS PLC control unit of the Panasonic brand, which is programmed to execute the actions at the corresponding times. The PLC activates the device or source that supplies the air and a series of solenoid valves that allow the passage of air to the mother tubes that are responsible for distributing it to the containers, and each container contains a filter at the inlet of sterile air. By enabling the first solenoid valve, air flows through a mother tube that connects with the containers containing the liquid nutrient culture medium. These containers begin to fill with air, which causes the medium to move towards the other interconnected container, which contains the explant.

When the explant bathing time is over, the second solenoid valve is activated, air flows through the other mother tube, and the liquid nutrient culture medium returns to its original container. This cycle is repeated several times according to the indications of the investigator or person in charge (frequency of transfers). This point is considered key for the good production of the plant, as is the medium used (nutritive culture solution). For the development of the explants, there are side lights to help illuminate the explants from time to time, which must be indicated on the HMI screen.

The proper functioning of the system depends on many variables. As mentioned above, the frequency of transfers, the medium used, the immersion time, volume of the medium, and type and number of explants are some. These variables change according to the species or variety of plant to be used or that is being evaluated, so they are mostly studied by researchers before starting a production cycle. However, the device also allows this part of the study, since by containing 4 modules that work in parallel, the researcher can carry out tests with four different media on the same species or variety, or on the contrary, 4 varieties of a species with the same medium [14]-[15].

IV. CONCLUSIONS

An automated temporary immersion system for the in vitro propagation of plant species has been designed and developed to meet the needs of planting material producers. This device is equipped with a mobile structure, allowing it to be repositioned easily according to the user's requirements. It features a robust general framework that supports the primary instruments of the propagation process. The system includes glass tanks that create an artificial environment conducive to plant micropropagation. Air access and distribution are managed through a pneumatic line, while a programmable logic controller oversees automatic process management. Electrical connections control each actuator, including electrovalves, and a lighting system regulates both the process and power supply. The user interface links user instructions with the programmable logic controller, and the process actuators facilitate the various stages of propagation, ensuring the selected plant materials are propagated under optimal health conditions.

V. CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Luis Ernesto Neira-Ropero: research, methodology, writing-original draft, writing-revision and editing.

Giovanni Orlando Cancino-Escalante: conceptualization, research, methodology, formal analysis, writing-original draft, writing-revision and editing.

Aldo Pardo-Garcia: supervision, validation, methodology, writing-proofreading and editing.

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