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Propuesta de diseño y automatización de biorreactor para sistemas de inmersión temporal

Bioreactor Design and Automation Proposal for Temporary Immersion Systems

Proposta de projeto e automação de biorreatores para sistemas de imersão temporária

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Resumen

En este trabajo se presenta un prototipo de biorreactor como alternativa para la micropropagación de especies vegetales. Se trata de una propuesta que disminuye de manera significativa los costos tanto en la construcción del biorreactor como en la técnica de propagación. La automatización de varios de sus elementos de manera intrínseca supone la posibilidad de ser más eficiente en su operación, lo cual podrá permitir incrementar la producción masiva de propágulos de diferentes especies agrícolas.

En la construcción de este nuevo sistema se utilizaron varios materiales: el uso de un contenedor para la sujeción de los elementos que participan en la inmersión, un motor a pasos, una plataforma que contendrá las plántulas, una placa electrónica Arduino Uno y una placa Raspberry Pi. Mediante la programación de la placa Arduino Uno, se automatiza el motor a pasos, el cual controla los movimientos de la plataforma de soporte que contiene los explantes. La construcción en general del sistema se logra por medio de materiales de fácil adquisición como plásticos y metales de bajo costo.

Como resultado, se logró construir un nuevo biorreactor para la micropropagación masiva de especies vegetales de fácil manejo, gracias al cual se espera tener una mayor producción a corto plazo.

Palabras clave: automatización, biorreactores, cultivo de tejidos, innovación, micropropagación.

Abstract

This work presents a proposal of biorreactor as an alternative for the micropropagation of plant species more economical than those existing in the market. The automation of several of its elements in a intrinsic way supposes the posibility of being more efficient in its operation, which may allow to increase the massive production of propagules of different agricultural species.

In the construction of this new system were used various materials such as: the use of a container to hold the elements involved in the dive, stepper motor, platform that will contain seedlings and board Arduino Uno. By programming the Arduino Uno board, it is possible to automate the motor in steps, which controls the movements of the support platform containing the explants. The general construction of the system is achieved by means of materials of easy acquisition like plastics and metals of low cost.

As a result, it was possible to build a new bioreactor for the mass micropropagation of low-cost, easy-to-use plant species, with which it is expected to have a higher production in the short term.

Keywords: automation, bioreactor, tissue culture, innovation, micropropagation.

Resumo

Neste trabalho, um protótipo de biorreator é apresentado como uma alternativa para a micropropagação de espécies vegetais. É uma proposta que reduz significativamente os custos na construção do biorreator, bem como na técnica de propagação. A automação de vários de seus elementos de forma intrínseca supõe a possibilidade de ser mais eficiente em sua operação, o que pode permitir aumentar a produção massiva de propágulos de diferentes espécies agrícolas.

Na construção deste novo sistema foram utilizados diversos materiais: o uso de um recipiente para a fixação dos elementos envolvidos no mergulho, um motor de passo, uma plataforma que conterá as mudas, uma placa eletrônica Arduino Uno e uma placa de Framboesa. Pi. Ao programar a placa Arduino Uno, o motor de passo é automatizado, que controla os movimentos da plataforma de suporte que contém os explantes. A construção geral do sistema é obtida por meio de materiais prontamente disponíveis, como plásticos e metais de baixo custo.

Como resultado, foi possível construir um novo biorreator para a micropropagação em massa de espécies de plantas de fácil manejo, graças às quais espera-se que tenha uma maior produção a curto prazo.

Palavras-chave: automação, biorreatores, cultura de tecidos, inovação, micropropagação.

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Introduction

Nowadays the crops exposed to the elements have had lower production than those cultivated through the implementation of specific technologies and systems. Because these implementations have managed to accelerate production and improve the quality of the product by having a regulated environment (Campos and Llanderal, 2003).

The contributions of computational sciences can be oriented to the care of crops through the programming of automatic tasks and through the observation or continuous revision of the crop in order to improve the production and quality of the crops (Rodríguez and Santana, 2015).

In recent decades, several horticultural and forestry species have been successfully propagated using bioreactors (Etienne and Berthouly, 2002). Bioreactors, either mechanical or pneumatic agitation, and those of temporary immersion, have been used to optimize the massive regeneration of somatic embryos, for example, in coffee; and they have also allowed a high degree of control over the cultivation conditions such as pH, aeration rate, concentration of oxygen, ethylene and carbon dioxide. The automation of the micropropagation process allows cost reduction (Etienne et al., 1997) and there are also great advantages in reducing intensive manual manipulation and increasing the rate of proliferation (Jones and Flores, 2007). In addition, crops can be initiated from several portions of plant tissue (Mallón, Covelo and Vieitez, 2011).

For the automation of a temporary immersion system (SIT) there are different applications and mechanisms that interfere in its control. The most common system is that where electrovalves are connected to a compressor with automatic ignition and silicone hoses that reach the bottom of the bioreactor, which controls its activation through a programmable timer to determine the frequency and duration of the immersion (Santos et al., 2011). Other recently developed systems include the use of open hardware and software platforms for automated control (Lugo, Arellano y Hernández, 2017).

With the information collected and in order to know the procedure of in vitro propagation of plant species through a SIT, we proceeded to conduct a field investigation in the Postgraduate School, Campus Montecillo. And thanks to this research, the needs of the personnel working in said institution were better known and a prototype was carried out in accordance with the requirements of the aforementioned personnel.

In the conventional SIT of type Automated Temporary Immersion Container (RITA®) on average a multiplication rate of up to 10 times is achieved during a crop cycle and depends on the species. With the new design that was announced, the production and quality of the outbreaks is expected to increase, without forgetting to follow the basic principles that are part of the in vitro propagation, such as the sterilization of all the material to be used and the preparation of the appropriate culture medium.

Figure 1 shows a SIT with RITA® type bioreactors where it is observed how filtered air is injected into the container and this causes the culture medium to rise to the compartment to bathe the tissues. This process is carried out depending on the times and frequencies of the dive.

Figura 1. SIT de tipo RITA en operación

Fuente: Elaboración propia

The objective of this work was to design a new low cost bioreactor in its construction and operation, taking into account the principles of sterilization of all the materials that compose it and the automation of its operation through software.

It is important to point out that, in spite of all the advances that have been made in the area of in vitro cultivation and traditional methodologies of propagation, to date has not been able to satisfy one hundred percent the demand of plant species, because Normally gelled semi-solid culture media, high number of culture vessels, growth rooms with numerous shelves and more people dedicated to planting and maintenance by subculturing plants in the different phases of micropropagation are required. Therefore, this technology applies only to those species that have difficulty propagating by seed or are sick or there is the possibility of having a high economic return; in the rest of the cases, the production costs of vitroplants are so high that their commercial use is limited (Albarrán *et al.*, 2014).

Materials and methods

Although microbial contamination is an aspect that can not be controlled in its entirety, there are methods that make disinfestation a little more effective for the correct development of explants. One of them, and here considered the most effective, is the one that resorts to the autoclave (Ramírez et al., 2006). And for the selection of compatible materials with this method it was necessary to perform tests that guarantee its functionality. Thus, based on the resistance of each one, those that could tolerate temperatures higher than 121 ° C were used to eliminate the microorganisms that could be present in the materials themselves, without affecting their functionality and physical condition, since for the Proper performance of a bioreactor is essential sterilization and properly handle asepsis standards.

In addition, it is important to emphasize that the air that is supplied must also be sterile and, therefore, there must be no mechanical breakage that could allow the entry of microorganisms. Although it must be taken into account that there is no quantitative, safe and reliable method to measure the number or concentration of contaminants in the system. (Foundation for Agrarian Innovation, Ministry of Agriculture, 2009). So it is essential to have a hermetic system that seals perfectly to avoid the introduction of microorganisms that affect the correct development of the explants.

For the development of the prototype, a container with a capacity of 2.6 liters was chosen (see figure 2). In addition to its easy handling, portability and practicality, it has the following characteristics:

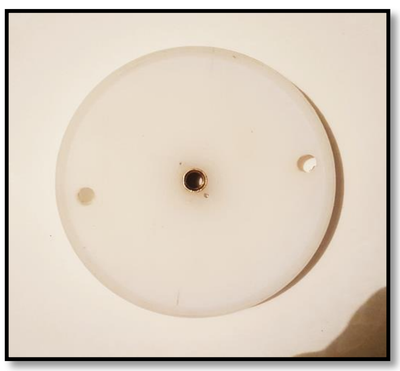
- The material with which it is manufactured is made of plastic resistant to autoclaving.
- Sealing cover 100% hermetic.
- Fully transparent container for the proper passage of light to the explants.

Figura 2. Contenedor de medio de cultivo

Fuente: Elaboración propia

To place the explants inside the container, a Nylamid platform was manufactured, which belongs to the family of polyamides (PA) nylon, with a circumference of four inches that fits perfectly inside the container (see figure 3).

Figura 3. Plataforma para explantes



Fuente: Elaboración propia

It also used a tool called a ball screw or computerized numerical control (CNC) eight millimeters (see figure 4). It is known as a CNC machine because a computer controls the position and speed of the motors that drive the axes of the machine (Of machines and tools, December 28, 2015). Once the machine is programmed, it executes all the operations on its own. This machine will help control the movement of the platform for its proper immersion into the culture medium.

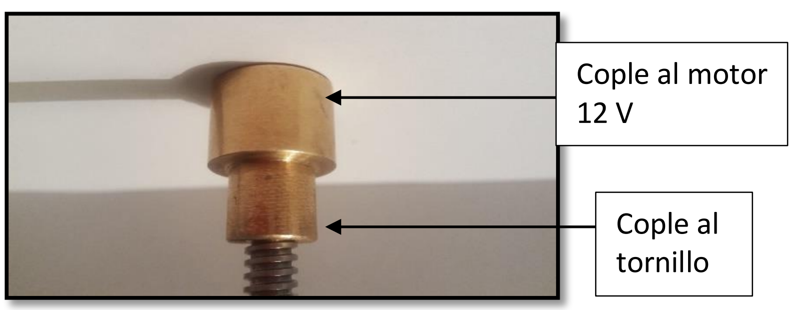
The CNC screw is placed in the middle of the Nylamid platform to perform the rotary movement and get the platform up and down inside the container.

Figura 4. Tornillo CNC de 8 mm

Fuente: Elaboración propia

A motor with steps of 12 volts was used to achieve the rotary movements that help the screw to rotate on its own axis. This motor was adjusted at the top. Thanks to the manufacture of a bronze coupling, the correct adjustment between the screw and the motor was made step by step. Figure 5 shows the perfect fit between both pieces, key aspect to achieve the ascending and descending movements of the platform that will contain the explants.

Figura 5. Cople para el motor a pasos



Fuente: Elaboración propia

The stepper motor together with its driver circuit known as the driver are responsible for controlling the rotary movement of the screw (see figure 6).

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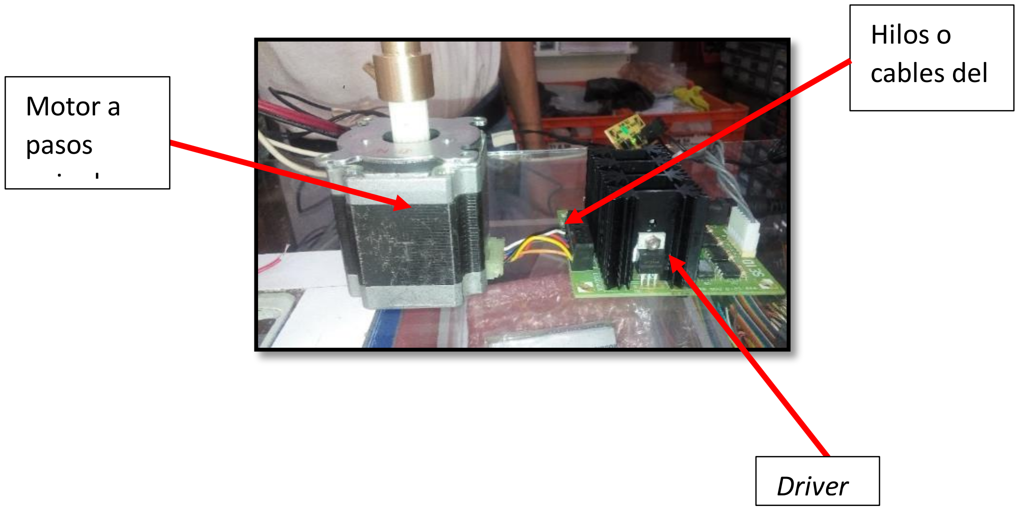
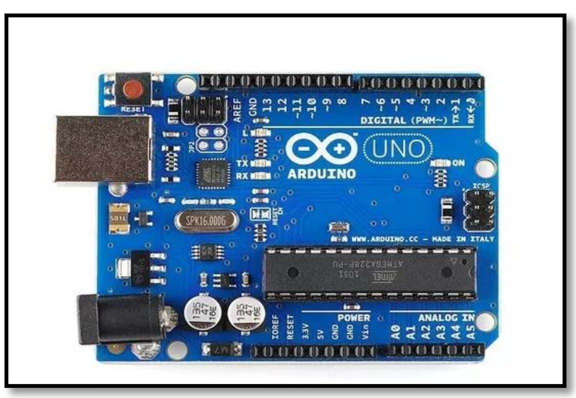
Figura 6. Motor a pasos de 12 V con *driver*



Fuente: Elaboración propia

The driver is not able to send pulses to the motor by itself. This requires an extra element that will contain the code to send the instructions to the driver and send the pulses to the motor. The control and manipulation of the elements is achieved thanks to the programming of an electronic platform called Arduino Uno (see figure 7). This, connected to the stepper motor driver, easily achieves the control of various electronic devices. To do this, pins 2-9 were used to connect and control the motor; and the output of 5V and ground (GND, by its equivalent in English) of Arduino to power its driver.

Figura 7. Placa Arduino Uno



Fuente: Elaboración propia

One of the characteristics of the RITA® system is that it must be permanently connected to a timer or computer equipment in order to perform the immersion functions. However, to partially dispense with this element it was decided to make use of a plate called Raspberry Pi, which is a low-cost reduced plate computer; it supports several necessary components in a common computer, it has several ports and inputs: two USB, one Ethernet and one HDMI output; these ports allow the minicomputer to be connected to other devices, keyboards, mice and screens.

It also has a chip that contains an ARM processor that runs at 700 MHz, a VideoCore IV graphics processor and up to 512 MB of RAM. In addition, it is possible to install free operating systems through an SD card. In summary, it is a minicomputer with which, if it is connected to a screen or monitor, the applications and programs it contains can be viewed (Sánchez, March 7, 2016). In figure 8 the plate is physically observed Raspberry Pi.

Figura 8. Placa Raspberry Pi con sus elementos

Fuente: Elaboración propia

To be able to handle the card, simulate a complete computer and develop the programs that are occupied, a microSD memory is required, which is responsible for containing the operating system to work correctly with the board. The operating system called Raspbian, on the other hand, allows to install the applications required for the operation of the bioreactor. Your desktop environment is shown in Figure 9.

Figura 9. Entorno de trabajo Raspbian

Fuente: Elaboración propia

The Arduino Uno board contains the movement code, together it is connected to the Raspberry Pi board, where the control application for the use of the biological researcher is located.

Software

For the automation of the described model, a part was developed with Arduino. This open-source electronic prototype platform (open source), based on flexible and easy-to-use hardware and software, is designed for artists, designers and anyone interested in creating interactive objects or environments (Enríquez, 2009).

Implementing a stepper motor with a CNC screw means that the operation is simpler than when using a compressor. Thanks to the Arduino board connected to the stepper motor driver, it is possible to control the screw that is inside the container and perform the movement of the platform ascending and descending for the immersion. However, the programming language of the Arduino board, in conjunction with the integrated development environment (IDE, for its acronym in English) of NetBeans, allow to program the immersion times of the explants to the culture medium.

The Arduino plate Uno board consists of electrical components where the main controllers that manage the other complements and circuits assembled in it are connected. However, a programming language is required to be able to start it up. This serves to control the various sensors that are connected to the board by means of instructions and parameters that are set from a computer.

The programming language of the Arduino board allows to program the immersion times of the explants to the culture medium. This programming language is free and has a large community of people who contribute ideas and efficient algorithms to solve the problems.

Once the motions of the motor have been programmed step by step in the IDE of Arduino, it is proceeded to program the time control for the correct immersion of the shoots in the culture medium. That is to say: the biological researcher will be able, in this way, to control the start date of the dive, the number of minutes the dive will last and the interval of time between one dive and another.

For the development of the desktop application, the Java programming language was used, which is a very popular language because it can be run on different platforms. There are different Java application development environments. This type of product offers the programmer an IDE to facilitate the complete process of application development: from the design, programming, documentation and verification of the programs (Martínez, 2012).

All the development code was made in the Netbeans IDE. Because it is a free development environment, you can make use of it and exploit all the attributes it grants for free, such as the process of controlling times and dates. Thanks to the libraries of NetBeans, then, it is possible to launch scheduled tasks without the need to perform calculations that would take more lines of code, in addition to being precise when executing the scheduled tasks.

Finally, the design of the interface was carried out in a simple way so that it could be intuitive for the user or for the biological researcher. There is only one screen, which is where the whole procedure will be performed. The user's only job, therefore, is to enter the data and start the system. The system will automatically run to perform all scheduled tasks (see figure 10).

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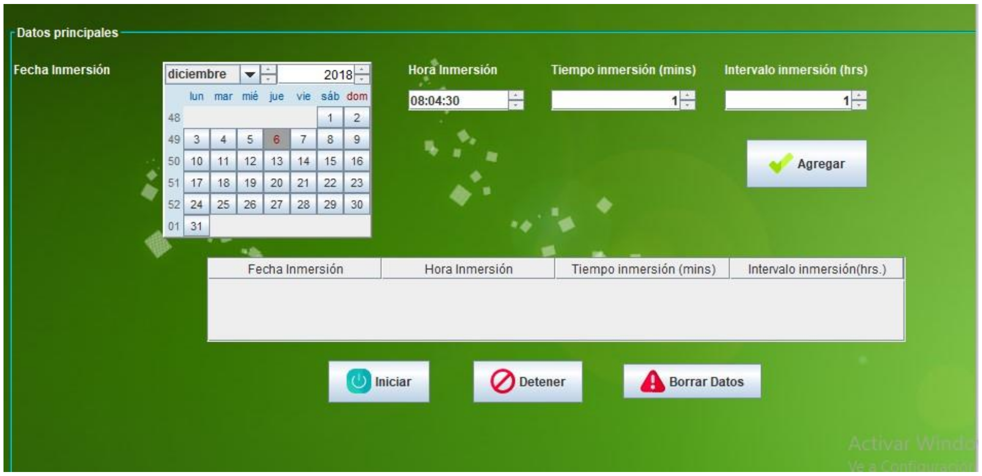
Figura 10. Interfaz de usuario



Fuente: Elaboración propia

It can be seen in Figure 10 that there are four important items; items that the user or the biological researcher can modify:

- Date of immersion: Enter the date on which the system starts the immersion of the explants.
- Dive time: The start time of the dives is specified.
- Immersion time: Specifies the time that the explants will be submerged in the culture medium (immersion time programmed in minutes).
- Immersion interval: Specifies the waiting time between one and another dive (waiting time in hours).

Results

The terminal prototype is presented below: body of the bioreactor and the part where all the necessary elements for the automation control of this are placed. Both the stepper motor and its driver and the Arduino Uno board are correctly connected for operation.

The individual automation by bioreactor suggests an advantage with respect to series systems: avoids possible contamination; It also has the possibility of connecting with several units at the same time to take advantage of the motor's capacity

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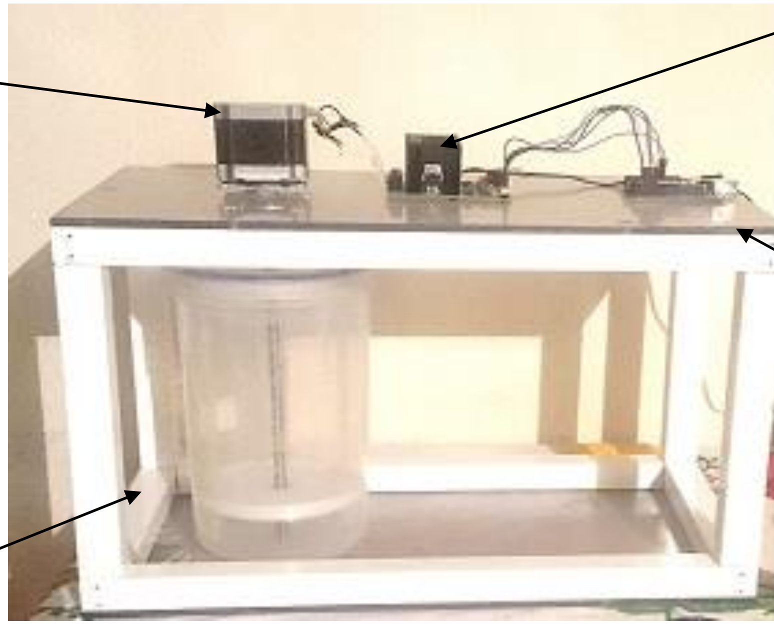
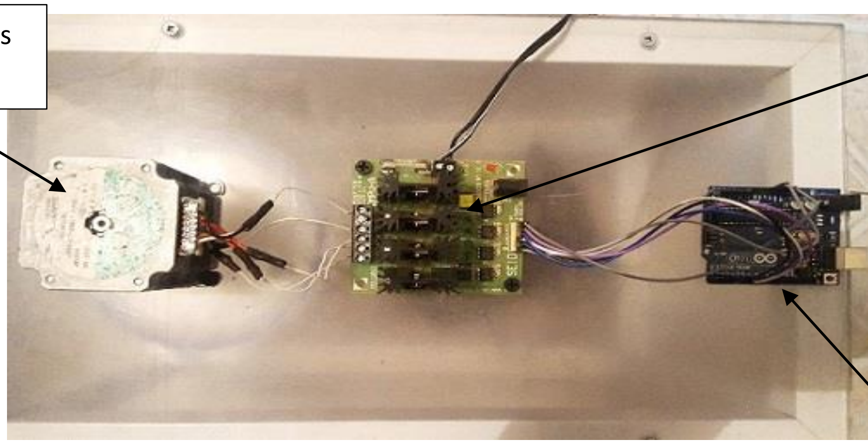
Figura 11. Biorreactor con sus elementos de control



Fuente: Elaboración propia

Figura 12. Elementos de control del nuevo biorreactor



Fuente: Elaboración propia

Conclusions

It was possible to build a prototype with resistant materials, low cost, easy to use, with parametric software that makes it adapt to any species to propagate, which allows exploring a more feasible alternative for the micropropagation of plant species. The desktop application, in addition, does not require special training for the staff or the biological researcher, since it can be easily manipulated and performed in the same way the necessary tasks for the immersion of the explants within the culture medium according to the needs of the plant material and the specifications indicated by the biological researcher.

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