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Strategy to improve the micropropagation of sugarcane plants using carbon dioxide injection

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Citation: Malagón-González F., Rodríguez-Jarquin, J.P., Bello-Bello, J. J., & Martínez-Sibaja, A. (2024). Strategy to improve the micropropagation of sugarcane plants using carbon dioxide injection. *Agro Productividad*. https://doi.org/10.32854/agrop.v17i10.2977

Academic Editor: Jorge Cadena Iñiguez
Associate Editor: Dra. Lucero del Mar Ruiz Posadas
Guest Editor: Daniel Alejandro Cadena Zamudio

Received: July 16, 2024.
Accepted: September 15, 2024.
Published on-line: November 08, 2024.

Agro Productividad, 17(10). October. 2024. pp: 133-141.

ABSTRACT

Objective: To develop a strategy for the improvement of micropropagation of sugarcane plants in the central region of Veracruz, using carbon dioxide injection.

Design/methodology/approach: Intelligent control and micropropagation techniques are used in this development.

Results: Improved micropropagation of sugarcane plants using carbon dioxide injection is demonstrated using intelligent control techniques.

Findings/conclusions: With this controlled carbon dioxide injection system, research can be carried out to obtain the most approximate values to contribute to better growth and adaptability in sugarcane crops. This can guarantee shorter adaptation times and thus generate greater economic gains for the producers.

Keywords: Carbon dioxide, micropropagation, intelligent systems, intelligent control, temporary immersion systems.

INTRODUCTION

The sugarcane farmland in Mexico presents average yields of 70 tons per hectare (t/ha), with the Central Region of the country being the most productive, with a mean of 117 t/ha (SAGARPA, 2017). The crop is cultivated in 14 states of the Republic, among which Veracruz stands out as the main producer with more than 287,000 ha (Rangel-Estrada S. Eloísa *et al.*, 2016).

Micropropagation of sugarcane plants in the central region of Veracruz is a vital process for the local agricultural industry, given the economic importance of this crop in the zone. Micropropagation or *in vitro* cloning of sugarcane is an important tool for

massive propagation of vigorous plants that are free of pests and diseases (Jose Humberto Caamal Velázquez *et al.*, 2014); however, an integral strategy is required to improve this process in order to ensure the efficiency and quality of the plants produced. Several key strategies have been identified, such as careful selection of genotypes, optimization of culture mediums, rigorous environmental control, and implementation of advanced technologies. In addition, the importance of staff training and collaboration with research institutions is emphasized. These strategies, when applied jointly, have the potential of significantly increasing the quality and the efficiency of micropropagation of sugarcane plants in the region, which would benefit both the producers and the sugar production industry in general.

In the study conducted by Bello-Bello (2022), they use silver nanoparticles for micropropagation (NPsAG) as an alternative to improve the asexual propagation of plants and, with this, to prevent the contamination of explants, the asepsis of the culture medium, and the accumulation of ethylene. In laboratory studies, it has been shown that NPsAG at low concentrations have a dose-response effect on the plant development, known as hormesis. With this study, an alternative is generated for other applications using plant tissue culture (PTC) techniques.

In the study by Joseph Campos Ruiz (2020), *in vitro* culture is presented as an alternative to obtain plants massively in the short term and of high quality. With the aim of providing a mechanism that supplies plants for the establishment of crops, the micropropagation of three sugarcane crops was evaluated (Isidro Elías, 2020). The study conducted by Fabián Contreras-Loera (2021) had the objective of developing an efficient system for the micropropagation of caper bush (*Capparis spinosa* L.), woody shrub of great interest for its products, notable resistance to drought, and tolerance to high temperatures. The research carried out by Reyna Rojas-García (2021) had the objective of establishing a protocol for the in vitro propagation of oregano. The establishment was achieved with nodal segments in MS basal medium; in this stage 57.32% of axenic explants were recovered.

Figure 1 shows the 5 phases of the micropropagation stage; this case study is based on phase (d), where the injection of carbon dioxide is conducted, which is essential for the healthy growth and development of plants (Bello-Bello, 2022). The adequate supply of

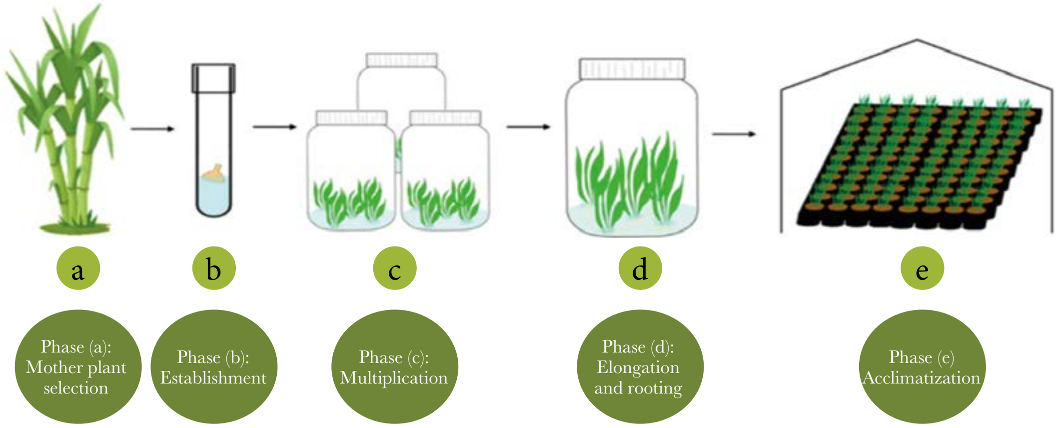

Figure 1. Stages of micropropagation applied in sugarcane crops (Bello-Bello, 2022).

CO_2 allows for plants to grow and develop optimally. A greater availability of CO_2 can increase the photosynthesis rate, which results in a faster growth and increased biomass production. The plants that have access to adequate levels of CO_2 tend to be more resistant to environmental stress, such as drought, high temperatures, or air pollution. This is because a higher photosynthesis rate provides the plant with more resources to combat stress and recover quickly.

Carbon dioxide is essential for the healthy growth and development of plants, since it is a key component in the photosynthesis process. It provides the raw material necessary for the production of carbohydrates, which are the plant's source of energy, and contributes to optimal growth, efficiency in water use, and to the plant's resistance to stress. The study conducted by Hiroki Gonome (2022) suggests an adaptive method to measure the plant's growth in real time by measuring the CO_2 consumption, which is resistant to environmental fluctuations. The study conducted by Xiadong Fan (2020), where the experimentation crop was peppers, examined the effects of a high CO_2 concentration on the growth of plants. The CO_2 provides the raw material for the photosynthesis process, improving the growth and productivity of the plants as consequence (Hesham A. Ahmed, 2020).

In the study performed by Santos (2011), the author mentions that for many intelligent control systems, the design methodology of the controller is essentially heuristic and based on certain principles of Artificial Intelligence or operative research. Among the definitions of Expert Systems, we find the following (Ana Lucía Sandoval Pillajo, 2021): One of the branches of AI is Knowledge-Based Systems (KBS), also called Expert Systems (ES). Within Artificial Intelligence, there are techniques of deep learning, particularly convolutional neural networks (CNN) (Juan C. Olguín-Rojas, 2022).

Advances in the fields of Automatic Control, Artificial Intelligence, Electronics, sensors, signal processing, actors, etc., provide new tools for complex system control (Santos, 2011). The system proposed by Wang (2022) describes in general the directions for the future enrichening in CO_2 for greenhouse production. The lack of CO_2 would not only result in a lower biomass, but rather the plants would also be of lower quality and resistance (Chowdhur, 2021).

MATERIALS AND METODOS

The study was conducted during the months of January to April, 2024, together with 3 institutions: Universidad Tecnológica del Centro de Veracruz (UTCV), Instituto Tecnológico Nacional de México campus Orizaba (ITO), and Colegio de Posgraduados campus Córdoba (COLPOS).

Materials and equipment

For the research project, the following materials and equipment were used:

- MIXER: Plastic container with capacity of 10 liters and maximum pressure of 10psi.
- SENSOR: The sensor used is a carbon dioxide sensor SN-300-LEDFL-CO2-N01-5000P, with measurement ranges from 0 ppm to 5000 ppm, with 24VDC feed and RS485 Modbus RTU exit.

- MICROCONTROLLER: The microcontroller used was an Aduino Mega card.
- ELECTROVALVES: 2-way 12VDC feed.
- OXYGEN TANK: The tank used has a capacity of 680 liters, with a regulator.
- CARBON DIOXIDE TANK: The tank used has a capacity of 680 liters, regulated at a pressure of 10 psi.
- HMI: The HMI used was brand KINCO GL070E, 7" in color, with connection to ethernet.

For the development of the system, a plastic recipient was used (mixer) with capacity of 10 L and pressure of 10 psi, where the carbon dioxide mixture was made. In this mixer, a SN-300-LEDFL-CO2-N01-5000P carbon dioxide sensor was adapted on the top part with measurement ranges of 0 ppm to 5000 ppm with 24VDC feed and RS485 Modbus RTU exit, which was connected directly to our microcontroller (Arduino Mega) in the TX and RX pines. With the processing of the signals in our microcontroller, the carbon dioxide injection was performed according to the parameters established initially; this injection was done through a 2-way 12VDC Electrovalves. An oxygen tank of 680 liters with regulator was used, as well as a carbon dioxide tank with capacity of 680 liters regulated at a pressure of 10 psi; the mixture to perform the injection was generated with these 2 tanks. Finally, an HMI of the brand KINCO GL070E of 7" in color was used, with connection to Ethernet, and this system was programmed with the KINCODTools software, where the RS485 communication was conducted.

Operation and functioning

Figure 2 shows the functioning diagram which is divided into 3 stages.

- Control stage: In the control stage, an HMI is used to establish the carbon dioxide values that the users with to have in each mixer. A microcontroller was used to perform the control and the electrovalves to regulate the flow. Compressed air is used for the mixture.
- Stage of mixture and sampling: In this stage, using the carbon dioxide sensors to measure the parts per million in each mixer, in case the desired parts per million are surpassed, oxygen is injected to decrease them.
- Stage of carbon dioxide injection: In this stage, the injection of carbon dioxide is performed, which is controlled by the electrovalves.

RESULTS AND DISCUSSION

Table 1 shows the results obtained after some time, attaining a total of 24 samples.

The correlation dispersion is proven in Trial 1, presented in Figure 3, where the dependent variable of CO_2 ppm monitored by the sensor is on the ordinate axis, while the seconds elapsed are on the abscissa axis, this being the independent variable.

With the data obtained that are presented in Table 1, calculations to obtain the linear regression equation were carried out.

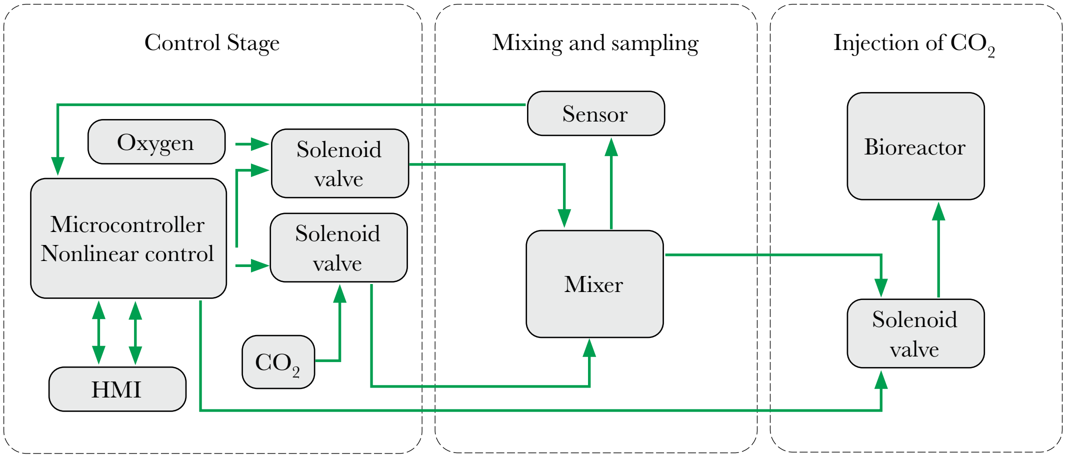

Figure 2. Functioning diagram of the carbon dioxide injection system.
Source: Prepared by the authors.

Table 1. SN-300-LEDFL-CO2-N01-5000P sensor performance data at P-1.

Parts per million (ppm) CO_2	**Time (s)**	**ppm*s**	**s^2**
23	33	2398	11881
22	109	2730	16900
21	130	3020	22801
20	151	3610	36100
19	190	4050	50625
18	225	4386	66564
17	258	4640	84100
16	290	5010	111556
15	334	5306	143641
14	379	5161	157609
13	397	5016	174724
12	418	4972	204304
11	452	4910	241081
10	491	4698	272484
9	522	4488	314721
8	561	4333	383161
7	619	4152	478864
6	692	3700	547600
5	740	3256	662596
4	814	2580	739600
3	860	1820	828100
2	910	958	917764
1	958	958	1006009
0	1003	0	1006009

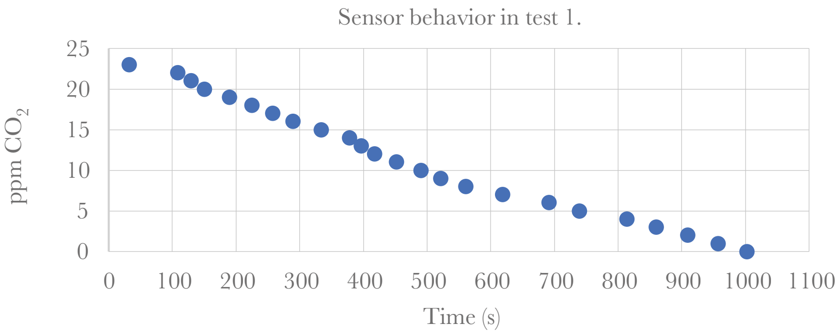

Figure 3. Dispersion of variables in Trial 1.

The simple linear regression is given by a straight line equation:

$$y = mx + b \tag{1}$$

The linear regression equation can be expressed as:

$$y = bx + a \tag{2}$$

Where: x=Seconds (s); y=Parts per million (ppm); n=Number of samples.

$$a = \frac{n\left(\sum xy\right)-\left(\sum x \sum y\right)}{n\sum X^2-\left(\sum X\right)^2} \tag{3}$$

$$b = \frac{\sum x - a\left(\sum x\right)}{n} \tag{4}$$

Substituting, there is:

$$a = \frac{24(85953)-(11536*276)}{24(7473874)-(11536)^2} \tag{5}$$

$$b = \frac{276-(-0.02421635091*11536)}{24} = 23.14 \tag{6}$$

Once the a and b values are available, they are substituted in equation 2, which results in the final correlation equation between x and y.

Substituting 6 in 2, there is:

$$y = -0.2421x + 23.14 \tag{7}$$

Where: -0.241 is the slope and 23.14 the y-intercept.

The correlation coefficient (r) of Trial 1 is obtained with the previous data. The following formulas were used to obtain the data in the table:

$$\sum\left(s-Ms^{\wedge}2\right)^{2}=1928903.333 \tag{8}$$

$$\sum\left(ppm-Mppm\right)^{2}=1150 \tag{9}$$

$$\sum\left(s-Ms\right)^{2}*\left(ppm-Mppm\right)^{2}=-46711 \tag{10}$$

We substituted 8, 9 and 10 in 11.

$$r=\frac{\sum\left(s-Ms\right)^{2}*\left(ppm-Mppm\right)^{2}}{\left(\sqrt{\sum\left(s-Ms\right)^{2}}*\left(\sqrt{\sum ppm-Mppm}\right)^{2}\right)} \tag{11}$$

Table 2. Data used to obtain the correlation coefficient in Trial 1.

s−Ms	(s−MS)^2	ppm−Mppm	(ppm−Mppm)^2	s−Ms*ppm−Mppm
−447.67	200405.44	11.50	132.25	−5148.17
−371.67	138136.11	10.50	110.25	−3902.50
−350.67	122967.11	9.50	90.25	−3331.33
−329.67	108680.11	8.50	72.25	−2802.17
−290.67	84487.11	7.50	56.25	−2180.00
−255.67	65365.44	6.50	42.25	−1661.83
−222.67	49580.44	5.50	30.25	−1224.67
−190.67	36353.78	4.50	20.25	−858.00
−146.67	21511.11	3.50	12.25	−513.33
−101.67	10336.11	2.50	6.25	−254.17
−83.67	7000.11	1.50	2.25	−125.50
−62.67	3927.11	0.50	0.25	−31.33
−28.67	821.78	−0.50	0.25	14.33
10.33	106.78	−1.50	2.25	−15.50
41.33	1708.44	−2.50	6.25	−103.33
80.33	6453.44	−3.50	12.25	−281.17
138.33	19136.11	−4.50	20.25	−622.50
211.33	44661.78	−5.50	30.25	−1162.33
259.33	67253.78	−6.50	42.25	−1685.67
333.33	111111.11	−7.50	56.25	−2500.00
379.33	143893.78	−8.50	72.25	−3224.33
429.33	184327.11	−9.50	90.25	−4078.67
477.33	227847.11	−10.50	110.25	−5012.00
522.33	272832.11	−11.50	132.25	−6006.83

Substituting, there is:

$$r = \frac{-46711}{(1388.849)*(33.9116)} = -0.9917811 \tag{12}$$

Trial 1 shows that the type of correlation is negative, since as the independent variable increases, the dependent variable will decrease; that is, as more time elapses, the ppm will be approaching zero. In obtaining the correlation coefficient, we can also see that the result is negative.

A second trial was conducted using 24 samples, which is presented in Figure 4, where we can see that there is a negative correlation, since as the independent variable increases, the dependent variable will decrease.

Through the results obtained from Trials 1 and 2, the coefficient of determination (R^2) can be calculated.

Coefficient of determination of Trial 1:

$$R^2 = 0.9836 \tag{13}$$

This value obtained is 98.36%, which is due to the dependent variable, that is, the gas monitored by the sensor, while the remaining 1.64% is because of other factors.

Coefficient of determination of Trial 2:

$$R^2 = 0.9953 \tag{14}$$

This value obtained is 99.53% of the dependent variable, that is, the gas flow monitored, while the remaining 0.47% is because of other factors such as the different day of the trial, the room temperature, the number of people within the room, or the sensor itself.

Figures 3 and 4 show the behavior of the sensor in face of the response to constant oxygen injections. As can be seen, the similarities between both graphs do not differ greatly one from the other. This indicates that when the sensor is subjected to different oxygen concentrations, as long as the flow is constant, it will continue to have coherent marking

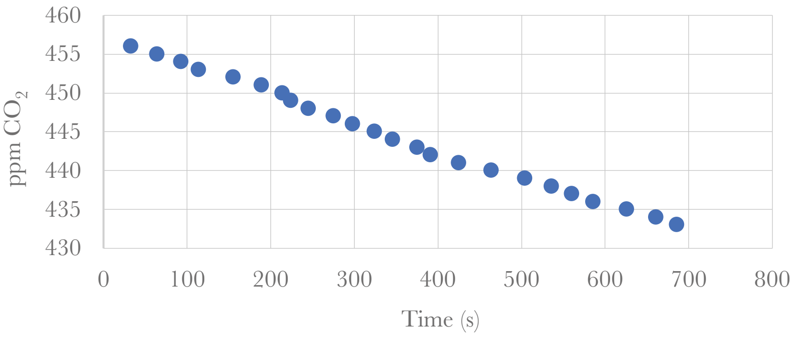

Figure 4. Dispersion of Trial 2 variables.

without considerable critical alterations for the system. With this, we can conclude that as long as the oxygen flow is kept constant, the parts per million of carbon dioxide will decrease depending on the time.

CONCLUSIONS

With the controlled carbon dioxide injection system, studies can be conducted to obtain the closest values to contribute to a better growth and adaptability in the sugarcane crops. This could guarantee shorter adaptation times and, with this, generate greater economic profits for the farmers.

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