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Evaluation of the effect of CO_2 concentration on the growth and yield of *Pisum sativum L.* using an automated greenhouse with fuzzy control

Evaluación del efecto de la concentración de CO_2 en el crecimiento y rendimiento de Pisum sativum L. mediante un invernadero automatizado con control difuso

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Abstract: The objective of this research was to evaluate the influence of CO_2 concentrations on morphometric and physiological variables, as well as on pea yield in Pamplona, North Santander. The research was conducted in an automated experimental greenhouse built at the University of Pamplona. One greenhouse was tested at a CO_2 concentration of 1300 ppm, while the other was tested at 600 ppm. Morphometric and physiological variables, including pod number, grain number, and grain weight, were measured. Significant increases were observed in height, leaf number, number of lateral shoots, and leaf surface area, but not in stem diameter and chlorophyll index. Higher CO_2 concentrations led to an increase in leaf area in pea plants, which was reflected in a higher pod number, grain number, and grain weight.

Keywords: automated greenhouse, fuzzy control, CO_2 concentration, C3 crop, *Pisum sativum L.*, climate scenario simulation.

Resumen: El objetivo de la investigación fue evaluar la influencia de la concentración de CO_2 sobre las variables morfométricas y fisiologías, y el rendimiento de la arveja en Pamplona Norte de Santander. La investigación se desarrolló en un invernadero experimental automatizado construido en la Universidad de Pamplona. Se evaluó una concentración de CO_2 de 1300 ppm y en el otro de 600 ppm. Se midieron variables morfométricas y fisiológicas, número de vaina, número de granos y el peso de los granos. Se verificaron incrementos significativos en la altura, número de hojas, número de brotes laterales y de la superficie de las hojas, no así del diámetro del tallo y el índice de clorofila. Una mayor concentración de CO_2 influyó en un aumento del área foliar de las plantas de alverja lo que se reflejó en un mayoro número de vainas, numero de grano y peso de los granos.

Palabras clave: invernadero automatizado, control difuso, concentración de CO_2, cultivo C3, *Pisum sativum L.*, simulación de escenarios climáticos.

1. INTRODUCTION

Climate is one of the main factors regulating the distribution of plant species, either directly or through physiological limitations on growth and reproduction, or indirectly through ecological factors such as competition for resources [1].

Climate change affects agricultural ecosystems in diverse ways, impacting crop development, the spread of pests and diseases, and crop yields [2]. It has been proposed that a significant increase in temperatures and changes in precipitation patterns caused by climate change in the tropical Andes will likely affect the size and distribution of glaciers and wetlands, depending on their ecosystem integrity and the availability of water for human consumption, irrigation, and energy production [3], [4].

Atmospheric CO_2 concentration has increased to levels between 370–380 ppm since the pre-industrial era [5], and climate change projections indicate that it could reach approximately 550 ppm by 2050 and about 730–1020 ppm by 2100 [6]. It is suggested that a doubling of CO_2 levels would increase global temperature by 1.5–4.5 °C, which would have a significant impact on plant life cycles, ecosystems, and socioeconomic systems in all regions of the planet [7].

With regard to research related to climate change and policy development in Colombia, it is argued that, due to the degree of uncertainty, research on climate modeling under different scenarios should be continued at detailed geographic scales and over the long term, in order to understand the extent to which different systems, regions, and crops are affected. The results of such studies would enable scientists and policymakers to develop appropriate adaptation plans. Therefore, further research is needed to develop climate-resilient, pest-resistant, and disease-resistant crops, making the preservation of agrobiodiversity and genetic resources crucial for this purpose [8].

Pea (*Pisum sativum* L.) is cultivated as a dry grain or as a fresh vegetable for human consumption, using its seeds. Canada, Russia, China, the USA, India, and Australia are the countries with the largest planted areas worldwide [9]. In 2015, 30,907 hectares of pea were planted in Colombia, of which 24,481 hectares were harvested. From these, 100,548 tons of green pod pea were obtained, with average yields of 4.1 tons per hectare per year. The department of Nariño was the main producer, with 58,401 tons, followed by the departments of Cundinamarca and Boyacá [10].

It is stated that in plants such as pea, whose metabolism is C3, CO_2 diffuses through the stomata and intercellular air spaces and ultimately reaches the chloroplast. Carbonic anhydrase catalyzes the reversible hydration of CO_2 to HCO_3^- in the aqueous phase, that is, in the chloroplast, cytosol, and plasma membrane, and is believed to maintain the CO_2 supply [11]. Kumar et al. (2020).

Plants have the capacity to capture atmospheric CO_2 and, through photosynthetic processes, metabolize it to produce sugars and other compounds required for their development (biomass). When biomass decomposes, it becomes part of the soil (in the form of humus) or is released as CO_2 (through the respiration of the microorganisms that process it). However, agricultural activities are also associated with a series of other CO_2 emissions, which we will refer to as “direct” and “indirect.”

Direct emissions are those generated during the cultivation process as a result of the fuel used in agricultural operations, as well as nitrogen oxides released from the soil due to fertilization (it should be recalled that the global warming effect of N_2O is 310 times greater than that of CO_2). Indirect emissions arise from electricity consumption and from the energy required for the manufacture and maintenance of agricultural equipment, the production of seeds or seedlings, and the manufacture of inputs. For this reason, it is necessary to determine the net CO_2 balance by subtracting direct and indirect emissions from the amount fixed by the plant, which makes it possible to determine whether different crops act as CO_2 sources or sinks [12].

At the University of Pamplona, Norte de Santander, Colombia, a project is being carried out to evaluate the influence of CO_2 concentration on yield components and on the physiology of different crops under greenhouse conditions with a controlled atmosphere, simulating future scenarios. In this way, it is possible to verify how C3 or C4 plants could benefit from or be affected by increased CO_2 concentrations. Based on this background, the following research question arises: To what extent

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does CO_2 concentration influence the growth and physiological processes of a C3 plant such as pea (*Pisum sativum* L.)?
Therefore, the objective of the present study was to evaluate the influence of CO_2 concentration on morphometric and physiological variables during the initial stage of development of pea (*Pisum sativum* L.) under greenhouse conditions.

2. METHODOLOGY

The research was conducted in the first half of 2023 at the main campus of the University of Pamplona, Colombia

2.1. Implementation of an automated greenhouse.

An experimental greenhouse was designed and built by the Automation Research Group as part of the University of Pamplona's Internal Call project, which allowed for the simulation of future scenarios and the study of the effects of climate change on crops.

2.2. Measurement of morphometric, physiological and productive variables in pea cultivation.

An experimental design was developed under controlled conditions with two treatments: one with the current CO_2 concentration (approximately 600 ppm, $\mu mol \cdot mol^{-1}$) and another with a concentration higher than the present level (1300 ppm), taking into account projections of future scenarios for the year 2100 [6], [13]. This independent variable was manipulated at these levels, while the remaining variables were controlled and regulated in both airtight compartments to maintain similar values.

CO_2 was generated through the combustion of Liquefied Petroleum Gas (LPG) using an innovation developed by the Automation and Control Group. It was stored and supplied to the greenhouse in gaseous form in the required amounts from this storage system and regulated by means of different valves. To measure CO_2 concentration, an SENO220 sensor was used, which allows readings in the range of 0 to 60,000 ppm.

Efforts were made to keep the remaining temperature conditions similar in both compartments using temperature controllers. Temperature regulation was achieved with a 900 BTU air-conditioning unit, which could increase or decrease temperature based on sensor readings and the coupled devices (reversing valve).

Relative humidity was controlled in the compartment with exchange with the external environment and regulated using equipment and sensors installed in both compartments, so that these variables were maintained at similar levels in each. Radiation conditions were similar in both compartments, as they had the same plastic covering on both the roof and the sides. Fans coupled with different sensors were used to maintain a uniform atmosphere within each airtight compartment.

All equipment was installed in a metal cabinet for system protection and safety, located 10 m from the greenhouse. The cabinet was equipped with its own power supply and a dedicated internet connection, which allowed the devices to be connected to the cloud and thus maintain a backup of the data collected by the installed sensors. This information was processed using different software tools (Arduino IDE, Visual Studio Code) and made available to the researchers to support decision-making for all activities, based on the control of the planned variables in the two greenhouse compartments, thereby enabling timely regulation. Historical data were downloaded from the cloud in .CSV format and then imported into Excel or Notepad in order to perform the corresponding analysis.

The experiment was conducted under the following conditions: average CO_2 concentration, temperature, and soil moisture up to 42 days after sowing the pea crop (Table 1).

***Table 1:** Average conditions of the variables during the time the experiment lasted.*

Variables	Cubicle 1 High concentration of CO_2	Cubicle 2. High concentration of CO2
CO_2 concentration (ppm)	1251.0	606.2
Soil Moisture (%)	72.5	71.8
Air temperature (°C)	19.3	19.5

As a substrate for the pea plants, soil from the A horizon of a site near the UP swimming pool was used, with which each trough was filled to three-quarters of its total volume (30 cm in height). Before starting the trial, the soil was sieved, and sampling and chemical analysis of the soil were performed. Soil correction and fertilization were applied to the entire required soil volume according to the corresponding recommendations [14]. Based on the chemical analysis of soil fertility, uniform fertilization was carried out throughout the soil volume in accordance with the nutritional requirements of pea. For example, nitrogen (200 mg dm^{-3}) was applied in the form of ammonium sulfate (20% N), and potassium (150 mg dm^{-3}) in the form of potassium chloride (60% K_2O).

The Santa Isabel variety was used. Botanical seeds were sown at a spacing of 20 cm between plants, 3 per planting site, and finally one plant per nest. The experimental planting took place in May 2023. Thirty plants remained in each cubicle. For sampling, measurements were taken from 12 plants in each treatment (2 per channel). The plants were managed agronomically according to the crop's Technical Standards [10].

Every seven days from June 13th, when the plants were 15 days old, the morphometric variables (height, stem diameter, number of leaves, leaf length and leaf width) were evaluated (Table 2):

Table 2: *How the variables were evaluated*

Variable	UM	Means for measurement
Seedling height	(cm)	Graduated ruler
Stem thickness	(mm)	Vernier Caliper
Emission of true leaves	(No.)	Physical count
Leaf length	(cm)	Graduated ruler
Width of the leaves.	(cm)	Graduated ruler
Leaf area	cm^2	Equation (Galindo and Clavijo, 2007).

The height was measured from the base of the plant to the first extended leaf using a measuring tape. The stem thickness was measured 1 cm from the base of the stem using calipers. The leaf area was estimated at 42 and 62 days after sowing (DAS) using the equation S= 0.6789 L*A [15].

At 95 DDS, the variables number of pods per plant and grains per pod were evaluated in 12 plants, and the grains of each of the 30 plants of each treatment were weighed.

The variables considered physiological for this objective were the total photosynthetic leaf area of the plant and the chlorophyll index. The total photosynthetic leaf area of the plant (TPLA) was estimated by multiplying the number of leaves per plant by the average leaf surface area or leaf area estimated in the previous section.

The chlorophyll intensity measurements of each plant in both treatments were performed using a chlorophyll meter. FieldScout CM 1000. Readings were taken at three levels of the plants: lower leaf, middle leaf, and upper leaf. The three values were averaged for each plant.

Comparisons were made for all morphometric variables, including average leaf area. The Mann-Whitney U test was used to compare data, as the distribution was not normal, with a 5% probability of error. The SPSS statistical package was used for analysis.

Leaf area and chlorophyll content were compared between plants using Student 's t-test with a 5% probability of error. The SPSS statistical package was used.

3. RESULTS AND DISCUSSION

3.1. Implementation of an automated greenhouse.

The greenhouse was constructed with two cubicles, each 2.5 m long and 2.5 m wide, and 2.5 m high (Figure 1). To ensure biosecurity, trap doors were installed to prevent increases and decreases in the variables. The greenhouse was then sealed with low density polyethylene, gauge 6 with UV additive (Figure 2).

Fig. 1. *Side view of the greenhouse*

Fig. 2. *Front view of the greenhouse*

To produce the CO_2 supplied to one of the greenhouse areas, the Automation and Control Group implemented an innovation: a CO_2 production system that burns Liquefied Petroleum Gas (LPG). The LPG was supplied to the greenhouse in gaseous form in the required quantities from storage and regulated by various valves. A SENO220 sensor, which allows readings from 0 to 60,000 ppm, was used to measure the CO_2 concentration (Figure 3).

To maintain similar temperature conditions in both cubicles and to measure the outside temperature,

temperature transducers were used (Figure 4) and (Figure 5). A 9000 BTU air conditioner was installed that could raise or lower the temperature by means of a reversing valve and according to the control signal from the embedded board.

Fig. 3. *CO $_2$ sensor brand SEN0220*

Fig. 4. *Rika330-02 brand humidity and temperature control.*

Fig. 5. *Rika330-01 brand humidity and temperature control.*

Relative humidity was controlled by air exchange between the cubicle and the environment, and was regulated with equipment and sensors placed in both cubicles to maintain similar levels in both. Since each cubicle had the same plastic roof and side covering, the radiation was similar. Additionally, fans were attached to the greenhouse structure at different locations to achieve atmospheric uniformity within each cubicle.

Blank tests of all the necessary controls were carried out before handing over the facility for the crop experiments, and an automated drip irrigation system was also installed, starting irrigation when the minimum of 50% field humidity was exceeded and closing the system at 60% humidity. Each section of the greenhouse had its own independent irrigation system and consumption per section could be measured weekly.

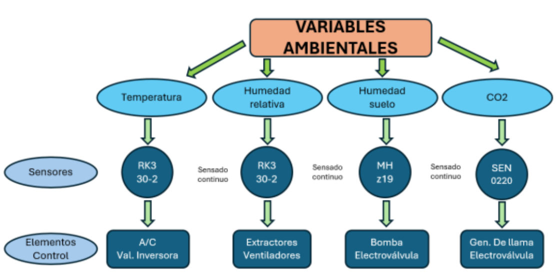

Fig. 6. *Reading and control scheme .*

3.2. Control Stage

The procedure for developing the PID and Fuzzy Control System is presented in Figure 6. A characterization of the greenhouse environment was carried out, identifying the environmental variables critical to crop health: temperature, relative humidity, soil moisture, and CO_2 concentration. The desired operating ranges for each variable were defined, taking as a reference technical literature and agronomic recommendations for ideal plant growth conditions. Then, reliable commercial sensors compatible with the data acquisition platform were selected (the sensors were calibrated and tested under controlled conditions before being integrated into the system) (Table 3).

Table 3: *Variables and recommended control.*

Variable	Recommended Control Type	Comments
Temperature	Mamdani -type Fuzzy Control with input: current temperature, error, and rate of change.	High sensitivity to disturbances; good fit with diffuse.
Relative Humidity	ON/OFF or diffuse control, with hysteresis.	Boolean or fuzzy logic can be used.
Soil Moisture	Supervised threshold control with fuzzy logic or rules	Irrigation control: it is not advisable to activate it for every small variation.
CO_2 level	Proportional control + logical rules (by ventilation or injection)	It does not require complex control, but it does require supervision.

3.2.1. Design and implementation of the fuzzy controller (Fuzzy Logic Controller)

The project was developed in a pre-existing greenhouse with controlled and protected environmental conditions and limited dimensions $V = 2.5m * 2.5m * 2.5m = 15.625m^3$. Due to its small size, the system exhibits low thermal inertia, requiring precise control to prevent abrupt temperature fluctuations. Data acquisition is performed using the industrial-grade RK330-02 sensor, known for its high stability and accuracy. Control logic processing is centralized on an Arduino Mega board, whose memory capacity allows for the management of fuzzy logic libraries and multiple peripherals. The power stage is managed by a Solid State Relay (SSR), which controls an air conditioning system with a reversing valve, enabling smooth transitions between heating and cooling modes. (Table 4).

The controller is based on two fundamental input variables that describe the thermal state of the system over time t: temperature error (e) (Eq. 1) and error variation Δe (Eq. 2). The former represents the instantaneous deviation from the setpoint, and the latter indicates the trend or rate at which the temperature changes, allowing the system to anticipate overshoots. The output (u) is a normalized signal between -100 and 100, where negative values activate the cooling cycle and positive values the heating cycle, both modulated by pulse-width modulation (PWM) via the SSR.

***Table 4:** Mathematical Parameters.*

Variable	Set	Guy	Parameters ([a,b ,c,d])
Mistake	MN (Heat)	Trapezoidal	[-10, -10, -5, -2]
	Z (Ideal)	Triangular	[-0.5, 0, 0.5]
	MP (Cold)	Trapezoidal	[2, 5, 10, 10]
Exit	AF (Cool)	Trapezoidal	[-100, -100, -70, -40]
	NULL	Triangular	[-10, 0, 10]
	CF (Warm up)	Trapezoidal	[40, 70, 100, 100]

3.2.2. System Characterization

Dimensions: $V = 2.5m * 2.5m * 2.5m = 15.625m^3$.

- RK330-02 Sensor: This is a high-precision sensor with a 4-20mA output, but an xy-IT05 module was used, which allows converting that current signal to a 0-5V voltage signal (compatible with Arduino Mega).
- Actuator: Air conditioner with reversing valve controlled by an SSR. This allows bidirectional control: Heating (Valve ON) and Cooling (Valve OFF), regulating the intensity by means of PWM or duty cycles on the SSR.

3.3. Fuzzy Controller Architecture

Mamdani -type inference model .

Mamdani -type fuzzy controller was designed with two input variables: temperature error (E) and the derivative of the error (dE). The output corresponds to the actuation level of the ventilation system, expressed in terms of the percentage of PWM applied to the actuator.

3.3.2. Input Variables

Error (∈) = Difference between the setpoint temperature (T_{set}) and the current temperature (T_{act}) (Eq.1).

$$e(t) = T_{set} - T_{act}(t) \qquad Ecu\ [1]$$

Error Variation (Δe) = Rate of change of temperature (Eq.1).

$$\Delta e(t) = e(t) - e(t-1) \qquad Ecu\ [2]$$

3.3.3. Output variables

Control Signal ( ) = μDuty Cycle Cycle) for the SSR.

- ✓ Range: [-100,100].
- ✓ Negative values: Cooling.
- ✓ Negative values: Heating.

3.4. Fuzzification (Fuzzy Sets)

crisp numerical values are transformed into degrees of membership in linguistic sets. Triangular and trapezoidal membership functions (Eq. 3) have been defined for the input variables, categorizing them from "Very Negative" (excessive heat) to "Very Positive" (excessive cold). The first step is to define the linguistic terms using triangular and trapezoidal membership functions due to their computational efficiency on Arduino.

For the Error (e):

- ✓ MN (Very Negative): [-10, -5, -2] (Very hot in the greenhouse)
- ✓ N (Negative): [-3, -1, 0]
- ✓ Z (Zero): [-0.5, 0, 0.5] (Ideal temperature)
- ✓ P (Positive): [0, 1, 3]
- ✓ MP (Very Positive): [2, 5, 10] (Very cold in the greenhouse)

For the Exit (μ):

- ✓ AF (Strong Cooling): [-100, -100, -60]
- ✓ AM (Moderate Cooling): [-70, -40, -10]
- ✓ NULL: [-10, 0, 10]
- ✓ CM (Moderate Heat): [10, 40, 70]
- ✓ CF (Strong Heat): [60, 100, 100]

3.5. Basis of Agronomic Rules

The logic is based on the thermodynamic behavior of the crop. If the error is positive (it's cold) and the error increases (it's getting colder), the response must be aggressive.

***Table 5:** Agronomic Rules.*

$\Delta e / e$	MN	N	Z	P	MP
N	AF	AF	A.M	NULL	CM
Z	AF	A.M	NULL	CM	CF
P	A.M	NULL	CM	CF	CF

3.6. Mathematical Formalism

Membership Level (μ)
For a triangular function defined by the points (a, b, c)(eq.3):

$$\mu_A(x) = \max\left(0, \min\left(\frac{x-a}{b-a}, \frac{c-x}{c-b}\right)\right) \quad Ecu\ [3]$$

Inference, aggregation, and defuzzification :
Mamdani inference , aggregation using the maximum operator, and defuzzification using the centroid method were employed (Figure 7).

Mambani Mechanism)

The system's intelligence resides in a decision matrix of 25 rules based on agronomic knowledge. The inference engine uses *Mamdani 's method*. For each rule i, the system evaluates the activation strength $\alpha_i$by applying the minimum operator (logical AND) (Eq. 4):

$$\alpha_i = min\left(\mu_{A_i}(e), \mu_{B_i}(\Delta e)\right) \qquad Ecu\ [4]$$

3.8. Defuzzification (Centroid)

To obtain an electrical signal applicable to the SSR, the aggregate of the output fuzzy sets is converted into a single real value. The Center of Gravity (COG) method is used, which calculates the point where the area under the curve of the aggregate membership function is balanced: Eq. 5:

$$u^* = \frac{\int \mu_{agg}(u) * u(du)}{\int \mu_{agg}(u)\, du} \qquad Ecu\ [5]$$

In Arduino, this is discretized as:

The signal $u^*$is processed by the Arduino Mega to execute the physical action (Eq. 6).

- Yeah$u^* > 0: Válvula\ inversora\ ON$
- Yeah$u^* < 0: Válvula\ inversora\ ON$
- Dead zone: A hysteresis range close to zero is established to prevent mechanical wear from unnecessary compressor switching.

$$u^* = \frac{\sum_{i=1}^{n} u_i * \mu(u_i)}{\sum_{i=1}^{n} \mu(u_i)} \qquad Ecu\ [6]$$

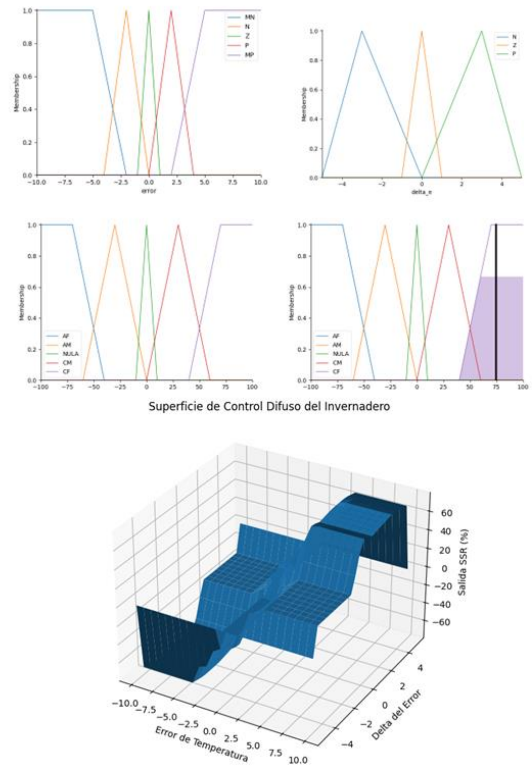

Fig. 7. *Range of error membership functions .*

4. MEASUREMENT OF MORPHOMETRIC, PHYSIOLOGICAL AND PRODUCTIVE VARIABLES IN PEA CULTIVATION

Pea plants showed greater height when subjected to a higher concentration of CO_2 for most of the experiment except at 42 DDS, at which point, despite the plants having a higher relative height value, no statistical difference was observed (Table 6).

Table 6: *Result of the statistical analysis of the height (cm) of pea plants subjected to different concentrations of CO_2 at different times of our.*

Treatments	Sampling (DDS)				
	14DD S	21DD S	28DDS	35DD S	42DD S
High $_{CO2}$	11.0 a	24.9 a	42.1 ns	57.8 a	86.8 a
Low $_{CO2}$	7.8 b	19.5 b	34.91 ns	51.5 b	68.7 a
Student 's t-test	3.8	2.4	1.7	2	6.5
P value	0.001	0.028	0.098	0.054	0

NS: No significant difference

*: Values followed by different letters in the columns indicate a significant difference according to the Student 's t- test for P<0.05

No influence of CO_2 concentration on the diameter of the pea plants measured at one centimeter above the ground from the beginning of the experiment until 42 DDS the plants (Table 7).

Table 7: *Result of the statistical analysis of the diameter of pea plants subjected to different concentrations of CO_2 at different times of our.*

Treatments	Sampling (DDS)				
	14DDS	21DDS	28DDS	35DDS	42DDS
High $_{CO2}$	3.00 to	3.00 NS	3.00 NS	3.00 NS	3.00 NS
Low $_{CO2}$	2.91 b	3.00 NS	3.00 NS	3.00 NS	3.00 NS
Student 's t-test	1.00	NP	NP	NP	NP
P value	,328	NP	NP	NP	NP

NS: No significant difference. NP: Statistical analysis was not performed.

Pea plants showed a greater number of leaves when subjected to a higher concentration of CO_2 at all sampling times (Table 8).

Table 8: *Result of the statistical analysis of the number of leaves of pea plants subjected to different concentrations of CO_2 at different times of our.*

Treatments	Sampling (DDS)				
	14DDS	21DDS	28DDS	35DDS	42DDS
High $_{CO2}$	9.83 a	21.50 a	32.83 a	53.66 a	74.50 a
Low $_{CO2}$	7.50 b	19.33 b	22.50 b	38.41 b	54.33 b
Student 's t-test	3,694	2,600	3,528	5,948	4,770
P value	0.001	0.016	0.002	0.000	0.000

*: Values followed by different letters in the columns indicate a significant difference according to the Student 's t- test for P<0.05

There was an advance in the emission of lateral shoots in the treatment with high CO_2 likewise a greater number of lateral shoots in the samples from 28 DDS to 42 DDS (Table 9).

Table 9: *Result of the statistical analysis Number of lateral shoots of pea plants subjected to different concentrations of CO_2 at different times of our.*

Treatments	Sampling (DDS)		
	28DDS	35DDS	42DDS
High $_{CO2}$	3.25 a	4.08 a	4.75 a
Low $_{CO2}$	0.25 b	3.00 b	3.75 b
Student 's t-test	13,519	3,767	4,506
P value	0.001	0.001	0.001

*: Values followed by different letters in the columns indicate a significant difference according to the Student 's t- test for P<0.05

CO_2 Concentration treatments in relation to the leaf surface area of the plants, maintaining a significant difference in all samplings carried out up to 35 DDS, at 42 DDS no difference was observed (Table 10).

Table 10: *Results of the statistical analysis of the leaf surface of pea leaves subjected to different concentrations of CO_2 at different times of our.*

Treatments	Sampling (DDS)				
	14DDS	21DDS	28DDS	35DDS	42DDS
High $_{CO2}$	3.38 a	4.39 a	5.10 a	5.28 a	5.45 NS
Low $_{CO2}$	3.17 b	3.58 b	4.30 b	4.54 b	5.34 NS
Student 's t-test	1,755	3,732	2,401	3,013	0.643
P value	0.093	0.001	0.025	0.006	0.527

NS: No significant difference

*: Values followed by different letters in the columns indicate a significant difference according to the Student 's t- test for P<0.05

$_{CO2}$ concentration treatments in relation to the total leaf area of the plants, maintaining a significant difference in all the samplings carried out at different times (Table 11).

Table 11: *Results of the statistical analysis of the total leaf area of pea plants subjected to different concentrations of CO_2 at different times of our.*

Treatments	Sampling (DDS)				
	14DDS	21DDS	28DDS	35DDS	42DDS
High $_{CO2}$	33.16 a	94.38 a	168.56 a	276.21 a	397.55 a
Low $_{CO2}$	23.27 b	69.82 b	98.68 b	163.80 b	291.44 b
Student 's t-test	4,804	4,234	3,876	5,285	3,570
P value	0.000	0.000	0.001	0.000	0.002

*: Values followed by different letters in the columns indicate a significant difference according to the Student 's t- test for P<0.05

The chlorophyll index showed no statistical difference in the treatments at any of the five sampling times (Table 12).

Table 12: *Results of the statistical analysis of the chlorophyll index of pea plants subjected to different concentrations of CO_2 at different times of our.*

Treatments	Sampling (DDS)				
	14DDS	21DDS	28DDS	35DDS	42DDS
High $_{CO2}$	141.77 NS	146.61 NS	214.52 NS	220.41 NS	138.20 NS
Low $_{CO2}$	137.30 NS	139.78 NS	167.96 NS	169.13 NS	135.31 NS
Student 's t-test	2,059	0.887	1,085	1,211	0.608
P value	0.052	0.385	0.290	0.239	0.549

NS: No significant difference

Plants subjected to a higher concentration of CO_2 achieved a significantly greater number of pods per plant and grains per pod than those maintained at low concentration, as well as a significantly higher grain weight production (Table 13).

Table 13: *Results of the statistical analysis for the number of pods subjected to different concentrations of CO_2 at different times of our.*

Treatments	Sampling (95 DDS)		
	No pods/ plant	No grains/ sheath	Weight of grains/ plant (g)
High $_{CO2}$	26.33 a	5.33 a	83.23 a
Low $_{CO2}$	16.58 b	4.66 b	41.06 b
Student 's t-test	9.03	2.27	9.82
P value	0.000	0.033	0.000

*: Values followed by different letters in the columns indicate a significant difference according to the Student 's t- test for P<0.05

5. DISCUSSION

The greater height observed in plants subjected to higher CO_2 concentrations is important, as this variable is relevant to pea development, since elongation allows the accumulation of nutrients produced through the photosynthetic process, which are then translocated to other parts such as leaves, pods, and grains. A positive response of this variable was verified, increasing proportionally with plant age, but to a greater extent in plants exposed to higher CO_2 concentrations.

The larger leaf surface area in plants subjected to higher CO_2 concentrations constitutes an important index for estimating the capacity of plants to intercept light, carry out photosynthesis, and achieve higher yields, as suggested by some authors [15]. In a study on the influence of *Trichoderma* concentration on pea development, plant height was measured and showed differences among treatments, which were later reflected in an increase in photosynthetic leaf area [18].

Considering the results of greater height, leaf surface, and leaf area at the initial stage of plant development, it is necessary to continue evaluations throughout the entire crop cycle to verify whether this finding is related to higher production and yield [15].

At none of the sampling times was a statistically significant difference observed between the two CO_2 concentration treatments with respect to pea plant stem diameter measured at 1 cm above the base of the stem. However, CO_2 concentration did influence a greater production of lateral shoots, which also contributed to a higher number of leaves, indicative of increased activity and stimulation of growth in pea plants subjected to high CO_2 concentration.

In this study, lateral shoots were observed earlier in the high-CO_2 treatment. The number of secondary stems emerged per plant has been considered in pea plants as part of biostimulation by *Trichoderma*, which was later associated with a greater number of leaves and increased photosynthetic area in some treatments [18].

The present results are consistent with those reported by other authors who compared and confirmed a 45% increase in biomass in C3 herbaceous species subjected to environments with high CO_2 concentrations [19].

The lack of differences in chlorophyll content is not surprising, as this frequently occurs in many experiments in which this parameter has been shown to be relatively stable, such as in a study conducted to evaluate pea varieties [20] or another aimed at assessing *Opuntia* varieties [21].

Considering the data obtained, it can be stated that an increase in carbon dioxide accelerates growth in pea plants. Plants combine this gas with water and light in the photosynthetic process, which enables glucose production. In this regard, results have shown that the leaf area of maize, a C4 species, evaluated at 80 days after sowing (DAS), was not affected by high CO_2 concentration (T-CO_2). In contrast, common bean, a C3 species, showed increases in leaf area under this condition—19% on the first sampling date and 25% on the second—although such increases were not statistically significant. These increases resulted from the development of at least one additional branch, as well as from a greater number and size of leaves in plants exposed to high CO_2 concentration, similar to what has been reported for soybean (*Glycine max* L.) [22], whereas no similar response was observed in tomato (*Lycopersicon esculentum*) [23].
Other studies report that an increase in CO_2 concentration to 800 ppm promotes greater biomass accumulation and leaf area growth in stevia (*Stevia rebaudiana* Bert.) [24].

These findings can be related to the potential for increasing crop yields under elevated CO_2 concentrations simulating future scenarios. This can be further verified in other crops, as the existence of an automated greenhouse at the University of Pamplona, with two compartments for comparing different CO_2 concentrations, provides an opportunity to continue studies such as the present one.

6. CONCLUSIONS

The implementation of an automated greenhouse for research purposes was achieved, simulating future scenarios for crops at the University of Pamplona. It was verified that a high CO_2 concentration caused significant increases in plant height, number of leaves, number of lateral shoots, and leaf surface area in pea cultivation at an early stage of development, but not in stem diameter.

A higher CO_2 concentration influenced an increase in the leaf area of pea plants during the initial stage of crop development, but had no effect on the chlorophyll index.

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