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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 CO2 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 CO2 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 CO2 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 CO2 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 CO2 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 CO2 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.

INTRODUCTION

Climate is one of the main factors regulating the distribution of plant species, either directly, 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, which impacts crop development, the spread of pests and diseases, and yield [2]. It is proposed that a significant increase in temperatures and changes in precipitation patterns caused by this climate change in the tropical Andes will probably affect the size and distribution of glaciers and wetlands depending on their ecosystem integrity, water available for human consumption, irrigation and energy production [3], [4].

The atmospheric CO_2 concentration has risen to levels between 370-380 ppm since the pre-industrial era [5], and climate change forecasts predict that ~550 ppm could be reached by 2050 and ~730-1020 ppm by 2100 [6]. A doubling of CO_2 levels is expected to raise temperature by 1.5-4.5 ºC, which would have a notable impact on the life cycle of plants, on ecosystems and on socioeconomic systems in all regions of the planet [7].

As for research on climate change and the policies to be followed in Colombia, it is proposed that, given the degree of uncertainty, research on climate modeling under different scenarios, at a detailed geographic scale and over the long term, should continue in order to determine the degree of impact on different systems, regions and crops. The results of such studies would allow scientists and policymakers to develop appropriate adaptation plans, so more research is needed to develop crops resistant to climate, pests and diseases, and therefore the preservation of agrobiodiversity and genetic resources is crucial for this purpose[8].

Peas (Pisum sativum L.) are a dried grain or fresh vegetable for human consumption by taking advantage of their grains; the countries of Canada, Russia, China, USA, India, and Australia are the countries with the largest planted areas worldwide[9]. In 2015, 30,907 hectares of peas were planted in Colombia, of which 24,481 hectares were harvested. Of these, 100,548 tons of green pod peas 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 the pea, whose metabolism is C3, CO_2 diffuses through the stomata and intercellular air spaces and finally reaches the chloroplast. Carbonic anhydrase catalyzes the reversible hydration of CO_2 to HCO_3 in the aqueous phase, that is, (chloroplast, cytosol and plasma membrane), and it is believed to maintain the supply of CO_2 [11]. Kumar et al. (2020).

Plants can capture atmospheric CO_2 and, through photosynthesis, metabolize it into sugars and other compounds they need for growth (biomass). When biomass decomposes, it becomes part of the soil (as humus) or is released as CO_2 through the respiration of the microorganisms that process it. However, agricultural operations also carry other CO_2 emissions, which we will call “direct” and “indirect”. Direct emissions are those generated during cultivation, from the fuel used in agricultural tasks and from the nitrogen oxides released from the soil by fertilization (recall that the warming effect of N_2O is 310 times greater than that of CO_2). Indirect emissions come from electricity consumption, the energy needed to manufacture and maintain 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 the direct and indirect emissions produced from the amount fixed by the plant, which will make it possible to determine whether the different crops are CO_2 emitters or sinks[12].

At the University of Pamplona, Norte de Santander, Colombia, a project is being developed to evaluate the influence of CO2 concentration on yield components and the physiology of different crops under greenhouse conditions with a controlled atmosphere simulating future scenarios, thus verifying how C3 or C4 plants could benefit from or be affected by higher CO2 concentration. From this background, the following question arises: To what extent does CO2 concentration influence the growth and physiological processes of a C3 plant such as peas (Pisum sativum L.)?

Therefore, the objective of the present research was to evaluate the influence of CO2 concentration on morphometric variables and physiologies in the early developmental stage of peas (Pisum sativum L.) under greenhouse conditions.

METHODOLOGY

The research was carried out in the first semester of 2023 at the main campus of the University of Pamplona, Colombia.

Implementation of an automated greenhouse.

An experimental greenhouse was designed and built by the Automation Research Group as part of the University of Pamplona Internal Call project, to make it possible to simulate future scenarios and study the effects of climate change on crops.

Measurement of morphometric, physiological and productive variables in the pea crop.

An experimental design was developed under controlled conditions with two treatments, one at the current CO_2

concentration (approximately 600 ppm, (µmol·mol^{-1}) and another at a concentration higher than the present one (1300 ppm), taking into account forecasts of future scenarios for 2100[6],[13]. This independent variable was manipulated at those levels, while the remaining variables were controlled and regulated in both hermetic chambers so that they stayed at similar values.

The CO_2 was produced by burning Liquefied Petroleum Gas (LPG) through an innovation developed by the Automation and Control Group; it was stored and supplied to the greenhouse in gaseous form in the necessary quantities from its storage and regulated by different valves. To measure the CO_2 concentration, a SENO220 sensor was used, which allows readings ranging from 0 to 60000 ppm.

Efforts were made to keep the remaining temperature conditions similar in both chambers by means of the temperature controllers. To regulate the temperature, a 900 BTU air conditioner was used, which can raise or lower the temperature based on the sensors and the coupled devices (reversing valve).

Relative humidity was controlled in the chamber with exchange with the environment and was regulated with equipment and sensors placed in both chambers so that these variables remained similar in both. The radiation in each chamber was similar, since they had the same plastic covering on both the roof and the sides. Fans coupled to different sensors were used to keep the atmosphere uniform inside each airtight chamber.

All the equipment was installed in a metal cabinet, for the protection and safety of the system, 10 m from the greenhouse; it had its own power supply and its respective internet point, which allows connecting the equipment to the cloud and thus having a backup of the data collected from the installed sensors. This information was processed with different software (Arduino IDE, Visual Code Studio) to make it available to the researchers and to support decision-making for all activities, based on control of the planned variables in the two greenhouse chambers and thus achieve timely regulation. The cloud histories were downloaded as .CSV files and then imported from Excel or Notepad, so that the relevant analysis could be performed.

The experiment was conducted under the following conditions: average CO_2 concentration, temperature and soil humidity 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	Chamber 1 High CO_2 concentration	Chamber 2. High CO_2 concentration
CO_2 concentration (ppm)	1251,0	606,2
Soil humidity (%)	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 pool was used, with which each channel was filled up to ¾ 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 carried out. Soil correction and fertilization were performed on the entire volume of soil required according to the recommendation in this regard[14]. Based on the chemical analysis of soil fertility, fertilization was applied to the entire soil volume uniformly, depending on the nutritional requirements of the pea. For example: nitrogen (200 mg dm ^{3}) 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 sowing distance of 20 cm between plants, 3 per sowing site, and finally one plant per nest was left. The experiment was sown in May 2023. Finally, each cubicle contained 30 plants. For sampling, measurements were made on 12 plants per treatment (2 per channel). The plants received agronomic management according to the crop's Technical Standards[10].

Every seven days starting on June 13, when the plants were 15 days after sowing, the morphometric variables (height, stem diameter, leaf number, leaf length and leaf width) were evaluated (Table 2):

Table 2: manner in which the variables were evaluated

Variable	M.U.	Measuring instrument
Seedling height	(cm)	Graduated ruler
Stem thickness	(mm)	Vernier caliper
True leaf emission	(No.)	Physical count
Leaf length	(cm)	Graduated ruler
Leaf width.	(cm)	Graduated ruler
Leaf area of the leaves	cm^2	Equation (Galindo and Clavijo, 2007).

Height was measured from the base of the plant to the first leaf spread with measuring tape. Stem thickness at 1cm from the base of the plant with a king's foot. Leaf area was estimated at 42 and 62 days after planting (DDS) using the equation S= 0.6789 L*A[15].

At 95 DAS, 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 as physiological in this objective were the total photosynthetic leaf area of the plant and the chlorophyll index. The total photosynthetic area of the plant (ATFP) was estimated by multiplying the leaf number per plant by the average leaf surface or leaf area of the leaves estimated in the previous section.

Chlorophyll intensity measurements of each plant in both treatments were performed using a FieldScout CM 1000 chlorophyllometer. Readings were taken at three plant levels: lower leaf, mid-level leaves, and upper level leaves. The three values were averaged for each plant.

Comparisons were made for all morphometric variables and mean leaf area of the leaves. Data were compared using the Mann-Whitney test, because of the lack of normality, with a 5% error probability. The SPSS statistical package was used.

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

RESULTS AND DISCUSSION

Implementation of an automated greenhouse.

The greenhouse was built with two cubicles 2.5 m long and 2.5 m wide, with a height of 2.5 m (Figure 1). To ensure biosecurity, trap doors were installed to prevent increases and decreases in the variables. The greenhouse was made airtight, lined with low-density polyethylene, gauge 6, with UV additive (Figure 2).



Fig. 1. *Side view of the greenhouse (Left). Fig. 2. Front view of the greenhouse (Right).*

For the production of CO_2 to be supplied to one of the greenhouse compartments, an innovation was developed by the Automation and Control Group, consisting of equipment for producing this gas by burning Liquefied Petroleum Gas (LPG), which was supplied to the greenhouse in gaseous form in the necessary quantities from its storage and regulated by different valves. To measure the CO_2 concentration, a SENO220 sensor was used, which allows readings from 0 to 60000 ppm (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. *Sensor for CO2 , SEN0220 brand (Left). Fig. 4. Humidity and temperature controller, Rika330-02 brand (Center). Fig. 5. Humidity and temperature controller, Rika330-01 brand (Right).*

Relative humidity was controlled by exchange between the cubicle and the environment, and was regulated with equipment and sensors located in both cubicles so that these variables were maintained similarly in both; the radiation in each cubicle was similar, since both had the same plastic covering on the roof and sides. In addition, fans coupled to the greenhouse structure were used at different locations, so as to obtain uniformity in the atmosphere within each cubicle.

Blank tests were performed on the entire operation of the necessary controls before delivering the installation 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 shutting off the system at 60% humidity. Each section of the greenhouse had its own independent irrigation system, and consumption could be measured per section weekly.

Figure 6 shows the procedure for developing the PID and Fuzzy Control System. We characterized the greenhouse environment

and identified the environmental variables that are critical for crop welfare: temperature, relative humidity, soil moisture and CO_2 concentration. Desired operating ranges were defined for each variable, using technical literature and agronomic recommendations for ideal plant growth conditions as a reference. We then selected reliable commercial sensors compatible with the data acquisition platform (the sensors were calibrated and tested under controlled conditions before being integrated into the system) (Table 3).

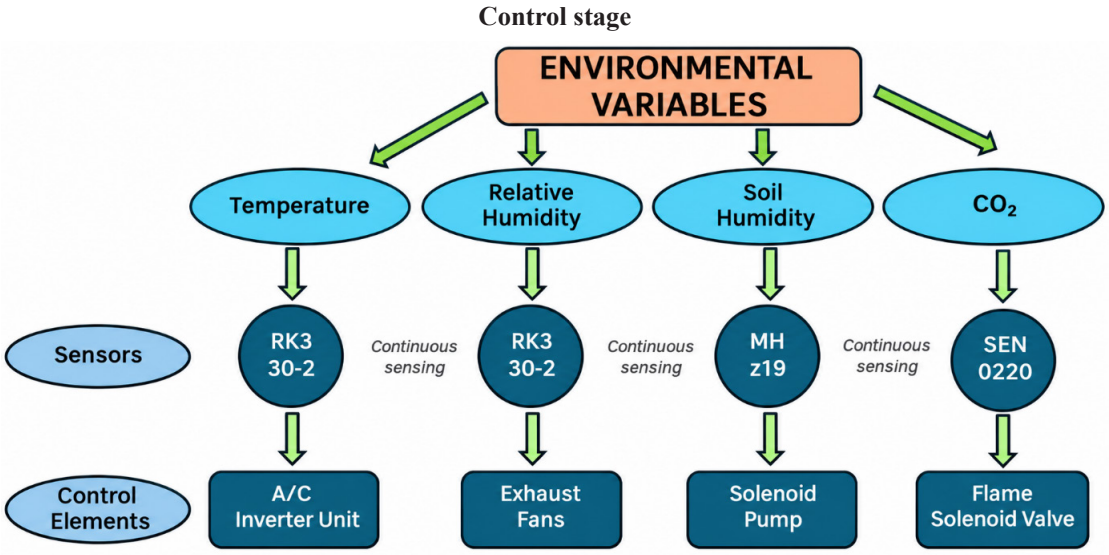


Fig. 6. *Reading and control scheme.*

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 adjustment with fuzzy.
Relative Humidity	ON/OFF or fuzzy control, with hysteresis.	Boolean or fuzzy logic can be used.
Soil Humidity	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 + logic rules (by ventilation or injection)	It does not require complex control, but it does require supervision.

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

The project was carried out in an already-built greenhouse for production under a regulated and protected environment with reduced dimensions $V = 2.5m*2.5m*2.5m = 15.625m^3$. Because of this low volume, the system has low thermal inertia, requiring precise control to avoid abrupt temperature fluctuations. For data acquisition, the RK330-02 industrial-grade sensor is used, recognized for its high stability and precision. Control logic processing is centralized on an Arduino Mega board, whose memory capacity allows managing the 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, allowing 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: the temperature error $^{(e)}$ (Eq.1) and the error variation Δe (Eq.2). The first represents the instantaneous deviation from the setpoint and the second indicates the trend or speed 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, modulated by pulse width (PWM) through the SSR.

System Characterization

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

Table 4: Mathematical Parameters.

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

• RK330-02 sensor: It is a high-precision sensor with a 4-20mA output, but an xy-IT05 module was used, which allows

converting that current signal into a 0-5V voltage signal (compatible with Arduino Mega).
- Actuator: Air conditioner with reversing valve controlled by 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.

Fuzzy Controller Architecture

Mamdani-type inference model.

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

Input Variables

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

$$e(t) = T_{set} - T_{act}(t)$$

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

$$\Delta e(t) = e(t) - e(t-1)$$

Output variables

Control Signal (μ) = Duty Cycle for the SSR.

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

Fuzzification (Fuzzy Sets)

At this stage, the "crisp" numerical values from the sensor are transformed into degrees of membership in linguistic sets. Triangular and trapezoidal membership functions (Ecu.3) have been defined for the input variables, categorizing them from "Very Negative" (excess heat) to "Very Positive" (excess 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] (Much heat 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](Much cold in the greenhouse)

For the Output (μ):

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

Agronomic Rule Base

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

Table 5: Agronomic Rules.

$\frac{\Delta e}{e}$	MN	N	Z	P	MP
N	AF	AF	AM	NULL	CM
Z	AF	AM	NONE	CM	CF
P	AM	NONE	CM	CF	CF

Mathematical Formalism

Degree of Membership (μ)

For a triangular function defined by the points (a, b, c)(ecu.3):

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

Inference, aggregation and defuzzification:

Mamdani inference, aggregation by means of the maximum operator and defuzzification using the centroid method were employed (Figure 7).

Inference (Mamdani Mechanism)

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

$$\alpha_i = min\left(\mu_{A_i}(e), \mu_{B_i}(\Delta e)\right)$$

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 aggregated membership function is balanced: Eq.5:

$$u^* = \frac{\int \mu_{agg}(u)*u(du)}{\int \mu_{agg}(u)du}$$

In Arduino, this is discretized as:

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

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

$$u^* = \frac{\sum_{i=1}^{n} u_i*\mu(u_i)}{\sum_{i=1}^{n} \mu(u_i)}$$

Measurement of morphometric, physiological and productive variables in the pea crop

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

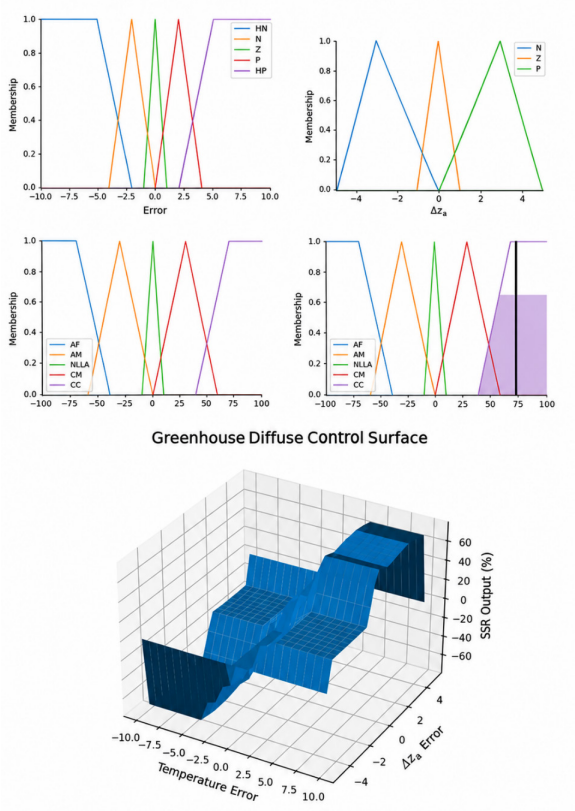


Fig. 7. *Range of membership functions of the error.*

Table 6: Results of the statistical analysis of the height (cm) of pea plants subjected to different CO2 concentrations at the different sampling times.

Treatments	Sampling (DAS)				
	14DAS	21DAS	28DAS	35DAS	42DAS
High CO_2	11.0 a	24.9 a	42.1 ns	57.8 a	86.8 a
Under CO_2	7.8 b	19.5 b	34.91 ns	51.5 b	68.7 a
Student's t	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 by the Student's t test for P<0.05.*

No influence of CO_2 concentration was observed on the diameter of thepea plants measured at one centimeter above the soil from the start of the experiment until 42 DAS (Table 7).

Table 7: Results of the statistical analysis of the diameter of pea plants subjected to different CO2 concentrations at the different sampling times.

Treatments	Sampling (DAS)				
	14DAS	21DAS	28DAS	35DAS	42DAS
High CO_2	3.00 a	3.00 NS	3.00 NS	3.00 NS	3.00 NS
Low CO_2	2.91 b	3.00 NS	3.00 NS	3.00 NS	3.00 NS
Student's t	1,00	NA	NA	NA	NA
P value	,328	NA	NA	NA	NA

NS: No significant difference. NA-Statistical analysis not applicable

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

Lateral shoots were emitted earlier in the high CO_2 treatment, and a greater number of lateral shoots was likewise recorded in the samplings from 28 DAS in the crop up to 42 DAS (Table 9).

Table 8: Results of the statistical analysis of leaf number of pea plants subjected to different CO2 concentrations at the different sampling times.

Treatments	Sampling (DAS)				
	14DAS	21DAS	28DAS	35DAS	42DAS
High CO_2	9.83 a	21.50 a	32.83 a	53.66 a	74.50 a
Under high CO_2	7.50 b	19.33 b	22.50 b	38.41 b	54.33 b
Student's t	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 by the Student's t test for P<0.05.*

Table 9: Results of the statistical analysis of the number of lateral shoots of pea plants subjected to different CO2 concentrations at the different sampling times.

Treatments	Sampling (DAS)		
	28DAS	35DAS	42DAS
High CO_2	3.25 a	4.08 a	4.75 a
Low CO_2	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 by the Student's t-test for P<0.05.*

A statistically significant difference was observed between the two CO_2 concentration treatments in the leaf area of the plant leaves, and a significant difference was maintained in all sampling events up to 35 DAS; at 42 DAS no difference was observed (Table 10).

Table 10: Results of the statistical analysis of the leaf area of pea leaves subjected to different CO2 concentrations at the different sampling times.

Treatments	Sampling (DAS)				
	14 DAS	21 DAS	28 DAS	35 DAS	42 DAS
High CO_2	3.38 a	4.39 a	5.10 a	5.28 a	5.45 NS
Under CO_2	3.17 b	3.58 b	4.30 b	4.54 b	5.34 NS
Student's t	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 within columns indicate a significant difference by the Student's t test for P<0.05.*

A statistical difference was observed between the two CO_2 concentration treatments in terms of total leaf area of the plants, and a significant difference was maintained in all sampling carried out at the different times (Table 11).

Table 11: Results of the statistical analysis of the total leaf area of pea plants subjected to different CO2 concentrations at the different sampling times.

Treatments	Sampling (DAS)				
	14DAS	21DAS	28DAS	35DAS	42DAS
High CO_2	33.16 a	94.38 a	168.56 a	276.21 a	397.55 a

Treatments	Sampling (DAS)				
	14DAS	21DAS	28DAS	35DAS	42DAS
Low CO_2	23.27 b	69.82 b	98.68 b	163.80 b	291.44 b
Student's t	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 did not show a statistical difference among treatments at any of the five sampling times (Table 12).

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

Table 12: Result of the statistical analysis of the chlorophyll index of pea plants subjected to different CO2 concentrations at the different sampling times.

Treatments	Sampling (DAS)				
	14DAS	21DAS	28DAS	35DAS	42DDS
High CO_2	141.77 NS	146.61 NS	214.52 NS	220.41 NS	138.20 NS
Low CO_2	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

Table 13: Results of the statistical analysis for pod number under different CO2 concentrations at the different sampling times.

Treatments	Sampling (95 DAS)		
	No. of pods/plant	No. of grains/pod	Grain weight/plant (g)
High CO_2	26.33 a	5.33 a	83.23 a
Under CO_2	16.58 b	4.66 b	41.06 b
Student's t	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 by the Student's t test for P<0.05.*

DISCUSSION

The greater height experienced by the plants subjected to a higher CO_2 concentration is important, since height is a relevant variable in pea development: elongation allows the accumulation of nutrients produced through photosynthesis, which are then transferred to other parts such as leaves, pods and grains. A positive response of this variable was verified; it increased proportionally with age, but to a greater extent in the plants subjected to a higher CO_2 concentration.

The greater leaf area of the leaves in the plants subjected to a higher CO_2 concentration is an important index for estimating the capacity of plants to intercept light, carry out photosynthesis and achieve higher yields, as some authors state[15]. In a study of the influence of the Trichoderma concentration on pea development, the height variable was measured; it differed among the treatments and was subsequently reflected in an increase in photosynthetic leaf area[18].

Given the results on greater height, leaf surface area and leaf area in the initial stage of the plants, evaluations need to continue throughout the entire crop cycle to verify whether this finding is related to obtaining greater production and yield[15].

At none of the sampling times was a statistical difference observed between the two CO_2 concentration treatments with regard to the diameters of the pea plants measured at 1 cm of stem height; however, the CO_2 concentration influenced a greater production of lateral shoots, which also contributed to a greater leaf number, indicative of greater activity and stimulation of the growth of the pea plants subjected to a high CO_2 concentration

In this research, lateral shoots were observed earlier in the high-CO_2 treatment. The emission of secondary stems per plant was taken into account in pea plants as part of the bio-stimulation by Trichoderma, which was subsequently related to the higher leaf number and the greater photosynthetic area of some treatments[18].

The present results are related to those of other authors who compared and verified a 45% increase in biomass in C3 herbaceous species subjected to environments with high CO_2 [19].

The lack of difference in chlorophyll contents is not surprising; this frequently occurs in many experiments where this parameter has proven not to be very variable, as in an experiment developed to evaluate pea varieties [20] or another to evaluate Opuntia varieties [21].

Taking into account the data obtained, it can be stated that the increase in carbon dioxide accelerates growth in pea plants. Plants combine this gas with water and light for the photosynthesis process, which enables glucose production. In this regard, there are results showing that the leaf area of maize, a C4 species, evaluated at 80 DAS, was not affected by the high CO_2 concentration (T-CO_2); in contrast, bean, a C3 species, showed increases in leaf area under this condition, of 19% on the first date and 25% on the second, although such increases were not significant. These increases were the result of the development

of at least one more branch, as well as a greater number and size of leaves in plants exposed to the high CO_2 concentration; similar to what was reported in soybean (Glycine max L.)[22], although in tomato (Lycopersicon esculentum) a similar response was not observed[23].

Other studies report that an increase in CO_2 concentration to 800 ppm promotes growth in biomass accumulation and leaf area for stevia (Stevia rebaudiana Bert.)[24].

The foregoing can be related to the possibility of increasing yield in the production of different plants with CO_2 concentrations simulating future scenarios, which can be verified for other crops since the existence of the automated greenhouse at the University of Pamplona with two chambers to compare different CO_2 concentrations is an opportunity to continue studies such as the present one.

CONCLUSIONS

The implementation of an automated greenhouse for research simulating future scenarios for crops at the University of Pamplona was achieved.

It was verified that a high CO_2 concentration caused significant increases in height, leaf number, number of lateral shoots and leaf surface area in the pea crop 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 at an initial stage of crop development, but not in the chlorophyll index.

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CONFLICT OF INTEREST

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