

Evaluation of the growth and quality of lettuce (*Lactuca sativa* L.) in a closed recirculating hydroponic system

Evaluación del crecimiento y calidad de lechuga (*Lactuca sativa* L.) en hidroponía con sistema cerrado de recirculación

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ABSTRACT

The production of lettuce in hydroponic systems with a recirculating nutrient solution has been growing, so it is necessary to evaluate the growth and quality of production under this system. Two harvest cycles were evaluated, comparing the behavior of physiological variables and growth rates on lettuce plants in a hydroponic system with a plastic cover. Lettuce plants were planted at 30 days after germination in an NFT hydroponic system. Nutrient solutions were prepared with sources of potassium nitrate, calcium nitrate, urea phosphate, magnesium sulfate and a source of minor *nutrified*. The second cycle had the highest total dry mass and leaf area index (LAI) at 43 days after transplant (dat). The relative growth rate (RGR) declined over time. The absolute growth rate (AGR) presented a sigmoid behavior as a gaussian bell shape; the leaf area index (LAI) increased until 43 dat, with the second cycle presenting the highest value; the net assimilation rate (NAR) decreased over time, with the second cycle having the highest value at 22 dat. The chlorophyll content for this variety was low, with a yellow pigmentation in the plant. The stomatal conductance (SC) in the two cycles at transplant time presented low values caused by the stress leaded by an imbalance in the pH of the solution, when the plants adapted to the system, this value increased.

Key words: RGR, AGR, NAR, SPAD, stomatal conductance.

RESUMEN

La producción de lechuga en sistemas hidropónicos con solución nutritiva recirculante ha venido tomando auge, por lo cual se requiere evaluar el crecimiento y la calidad de la producción bajo este sistema. Se evaluaron dos ciclos de cosecha comparando el comportamiento de variables fisiológicas e índices de crecimiento de plantas de lechuga en hidroponía bajo cubierta plástica. Las plantas de lechuga fueron sembradas a los 30 días de germinadas bajo el sistema de hidroponía NFT. Se prepararon soluciones nutritivas con fuentes de nitrato de potasio, nitrato de calcio, urea fosfato, sulfato de magnesio y una fuente de menores *Nutrified*. El segundo ciclo obtuvo la mayor masa seca total, índice de área foliar (IAF) a los 43 días después del trasplante (ddt). La tasa relativa de crecimiento (TRC) fue decreciendo con el tiempo. La tasa absoluta de crecimiento (TAC) presentó un comportamiento sigmoideo en forma de campana de gauss, el índice de área foliar (IAF) fue incrementando hasta los 43 ddt, siendo el segundo ciclo el que presentó el mayor valor, la tasa asimilación neta (TAN) disminuyó a través del tiempo, siendo el segundo ciclo el que mostró el mayor valor a los 22 ddt. El contenido de clorofila para esta variedad fue bajo por la pigmentación amarilla de la planta. La conductancia estomática (CE) en los dos ciclos al momento del trasplante presentó valores bajos causados por el estrés ocasionado por un desbalance en el pH de la solución, cuando las plantas se adaptaron bien al sistema se incrementó este valor.

Palabras clave: TCR, TAC, TAN, SPAD, conductancia estomática.

Introduction

In Colombia, the area harvested for lettuce in 2014 was 4,070.41 ha, with a production of 83,643.79 t (Agronet, 2014). The departments with the highest production were Cundinamarca, Antioquia, Nariño, Valle del Cauca, Norte de Santander and Boyacá. The Department with the highest yield was Nariño with 31.5 t ha⁻¹, while Boyacá presented

a yield of 16.1 t ha⁻¹ (Agronet, 2014), which demonstrates the need for more research in this crop.

Hydroponics use a system where nutrients reach plants through a nutrient solution, with different sources of fertilizers, providing the essential nutrients for the good development of plants (Kratky, 2005). Currently, hydroponic systems have become an intensive production system and

Received for publication: 22 March, 2017. Accepted for publication: 20 July, 2017

Doi: 10.15446/agron.colomb.v35n2.63439

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generally require high technology and high economic resources; their use has been successfully developed in many countries (Arcos *et al.*, 2011).

According to Sánchez del Castillo *et al.* (2014), the high costs of fertilizers and the environmental impact generated by their excessive use have increased the popularity of closed hydroponic systems, which capture and reuse drainage water, and reduce water and fertilizer consumption and the impact of the crop on the environment (Massa *et al.*, 2010). Sánchez del Castillo (2014) stated that some of the advantages found in the hydroponics system include high planting densities and an ideal balance of water and nutrients. The disadvantages are the high initial costs and extensive knowledge required for the functioning of these systems (Kotsiras *et al.*, 2016)

The technique of recirculating nutrient solution, known as NFT (Nutrient Film Technique), consists of the permanent flow of small amounts of solution through pipes, which allow the plants to take up the necessary nutrients for their adequate nutrition (Wortman, 2015). In NFT hydroponics, it is important to consider the following factors: maintain the temperature in the solution between 13 and 15°C, avoid a reduced absorption of the nutrients, pH must be in the range of 5.5 to 6.5, which applies to almost all plants, electrical conductivity (EC) should be around 1.5 to 3 mS cm⁻¹ and the channels should have a slope of 1.5% to 2% (Wortman, 2015). This has become a management option that has been practiced in lettuce (Massa *et al.*, 2010).

Therefore, the objective of this research was to evaluate the growth and quality of lettuce in a hydroponic system under a plastic cover in Tunja (Boyacá, Colombia) and with a recirculating nutrient solution in order to validate the benefits of this production system.

Materials and methods

The experiment was carried out in the mesh house of the Facultad de Ciencias Agropecuarias of the Universidad Pedagógica y Tecnológica de Colombia (UPTC), Tunja, located at an altitude of 2,690 m a.s.l. with coordinates 5°32'N and 73°23'W. Inside the plastic cover the average temperature was 17.5°C and the relative humidity (RH) was 71.6%. The laboratory analyzes were carried out in the Plant Physiology Laboratory of the UPTC.

Two culture cycles were performed for a comparison and verification of the data; data collection was done every 8 d after transplantation. A NFT hydroponic structure was built, with four PVC pipes, supported on a wooden structure; the pipe had a slope of 1.5% to facilitate the circulation

of the nutrient solution, which was collected and stored in a 500 L tank; the system was controlled with a timer and the pump was activated 20 min h⁻¹. The PVC pipes had 0.055 m diameter holes at a distance of 0.2 m, with a space between tubes of 0.3 m, for a capacity of 120 plants.

The nutrient solution was prepared based on the mg kg⁻¹ concentration proposed by FAO and HEWIT (Gilsanz, 2007). The sources used were potassium nitrate, calcium nitrate, urea phosphate, magnesium sulfate and minor nutrifeed® as a source of minor elements. The concentration in mg kg⁻¹ used in the test can be seen in (Tab. 1). pH monitoring was performed daily with a Hanna HI8424 potentiometer (Hanna Instruments, Woonsocket, RI, USA) and the electrical conductivity was measured every 4 d with an Oatkon CON 500 conductivity meter (Oatkon Computing, Australia); 10% KOH was used for pH adjustment.

For the test, 30 d-germinated *Lactuca sativa* L. var. Black Seed Simpson seedlings were used, which were transplanted to the PVC tubes, supported with polyurethane foam.

Six samples were taken, selecting eight plants at random, and the following variables were determined: fresh root and leaf mass with direct measurement using a 0.01 g precision Acculab VIC 612 electronic balance (Sartorius Spain S.A., Madrid, Spain); root and leaf dry mass after subjecting the samples to 75°C for 48 h. The leaf area was measured using the methodology proposed by Rincón *et al.* (2012). In addition, every third day, measurements were taken for the Chlorophyll Soil Plant Analysis Development (SPAD) units with a SPAD-502 Plus (Decagon Devices Inc., Pullman, WA, USA); the stomatal conductance (SC) was also determined with a Leaf Porometer SC-1 device (Decagon Devices Inc., Pullman, WA, USA).

TABLE 1. Description of the concentration of nutrients applied in the two cycles

Element	Concentration (mg kg ⁻¹)
N	223.86
P	56.88
K	238.71
Ca	149.85
Mg	81.65
S	112.38
Fe	4.00
Mn	2.00
Cu	0.16
Zn	0.16
B	0.24
Mo	0.08
Co	0.08

The temperature was recorded with an Extech RHT20 datalogger (Extech Instruments, Waltham, MA, USA) every 30 min in order to calculate the heat days degrees, using the formula used by Ardila *et al.* (2011), described below:

$$GD = \frac{T_{max} + T_{min}}{2} - T_{base} \quad (1)$$

Where T_{max} is the maximum air temperature, T_{min} is the minimum air temperature; T_{base} is the temperature at which the metabolic process of lettuce is minimal: this temperature was 5.5°C, as recommended by Gutierrez (2011).

For the analysis of the data, the growth models with the best fit were determined for each variable. Microsoft Excel 2010 and SAS v. 9.4e (Cary, NC, USA) were used.

TABLE 2. Description of the parameters that were measured

Index	Description	Formula	Unidades
LAI	Leaf area index	L_a/P	Adimensional
RGR	Relative growth rate	$(1/W)*(W2-W1)/(T2-T1)$	$g\ g^{-1}\ d^{-1}$
AGR	Absolute growth rate	$(W2-W1)/(T2-T1)$	$g\ d^{-1}$
NAR	Net assimilation rate	$(1/LA)*(W2-W1)/(T2-T1)$	$g\ cm^{-2}\ d^{-1}$

W = total dry mass (g); LA = leaf area (cm²); P = soil area (cm²); T = time.

Results and discussion

Leaf dry mass

The logistic model was fit to the total mass behavior, presenting a sigmoidal growth in the two cycles (Tab. 3); however, the second cycle developed better than the first, with a final dry mass accumulation of 16.5 g as compared to the first, which had an accumulation of 13.2 g (Fig. 1), these values are similar to those found by Valverde (2013) in lettuce variety Beyonce planted in an NFT system, where it

obtained a final average mass of 18.7 g. Possible differences in the dry matter accumulation in the two cycles probably occurred because of pH problems in the nutrient solution of the first cycle, which limited nutrient uptake and plant development.

In the first and second cycle, the linear phase of dry mass growth started from 22 and 15 dat to 43 dat, respectively. Generally, in bi-annual plants, three phases of growth can be seen: exponential phase, linear phase and senescence phase, where, in the first phase, growth is slow because the plants are in cell division; in the second phase, there is an increase in length and, in the final phase, the plant ceases growth because it is the maturation phase (Degiovanni *et al.*, 2010).

Growth is an irreversible increase in dry matter, which results in a quantitative increase in plant size and weight; for this process, plants perform metabolic differences that are referred to as sources and sinks (Ñustez *et al.*, 2009). Plant growth and dry matter accumulation are related to nutrient uptake and absorption; this process occurs only if the plant increases in size (Barraza, 2012).

According to Hernández and Soto (2012), plants subjected to higher temperatures during the day, generate a faster appearance of new leaves and, consequently, there is a greater rate to sunlight capture, which causes a greater accumulation of dry mass.

TABLE 3. Adjustment equations for first and second cycle total dry mass

Parameter	Logistic model	R ²
Total dry mass cycle 1	$Y = \frac{36.764}{1 + e^{-0.1762*(ddt-44.563)}}$	0.99
Total dry mass cycle 2	$Y = \frac{26.223}{1 + e^{-0.1527*(ddt-35.148)}}$	0.99

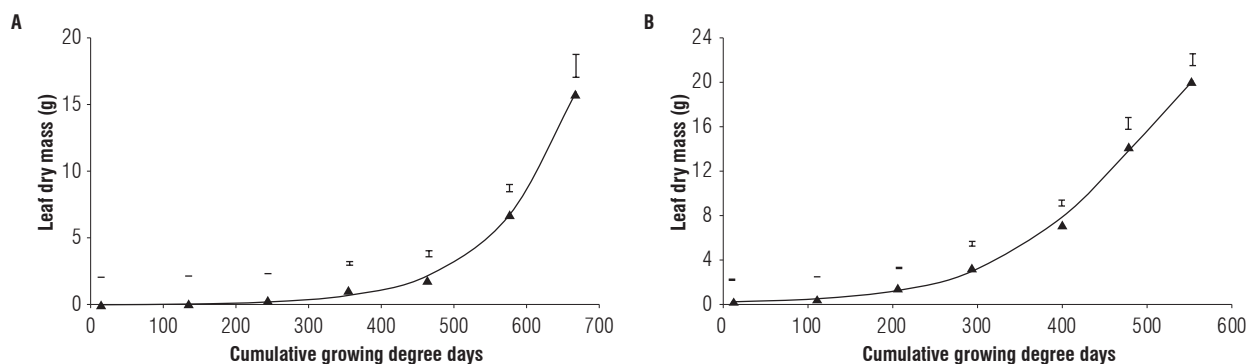


FIGURE 1. Behavior of leaf dry mass during development and growth of lettuce planted in a hydroponic system with a recirculating nutrient solution. A. First cycle; B. Second cycle. The vertical bars indicate the standard error ($n=8$).

Relative growth rate (RGR)

The highest RGR value occurred immediately after transplantation and subsequently decreased throughout the growth. This behavior was similar in both cycles. For the first and second cycle, the decline was very slow until 36 and 26 dat, respectively (Tab. 3), with a very marked slowdown in growth up to 43 dat. It was observed that, when the plants began to grow more rapidly, CRT decreased faster (Fig. 2A).

The RGR of the second cycle had a behavior similar to the lettuce Roman variety because, according to Martínez and Garcés (2010), 18 dat saw a significant decline, which began the phase of faster growth. In three varieties of gourmet lettuce, the RGR showed a weak negative correlation because an increasing dry mass meant the growth rate decreased (Quintero, 2015).

Absolute growth rate (AGR)

The AGR for the total dry mass in the first and second cycle showed a slow ascent until 15 and 7 dat, respectively, and then showed an accelerated increase until reaching its maximum value at 43 dat for the first cycle (1.58 g d^{-1}). The second cycle presented maximum growth at 36 dat (0.99 g d^{-1}), then began to decrease at 43 dat. The first

cycle obtained the highest AGR value, but was slower to start its acceleration, reflecting less accumulation of dry mass in leaves (Fig. 2B). In this regard, Hernández and Soto (2012) found that, in the dry mass of maize plants, the AGR showed a simple sigmoid growth in the form of a gauss bell, similar to that seen in lettuce cultivation. The gauss bell was not evident because the cycle finished at 43 dat. In plants, the AGR decreases in the vegetative organs with age because of the reduction of meristematic tissues (Granier and Tardieu, 2009).

Net assimilation rate (NAR)

In the two evaluated cycles, the NAR presented an increase up to 22 dat; the first cycle had a maximum value of $0.00080043 \text{ g cm}^{-2} \text{ d}^{-1}$ and the second one showed a value of $0.00082182 \text{ g cm}^{-2} \text{ d}^{-1}$, then began to decrease until 43 dat, just when the vegetative cycle ended (Fig. 2C). Generally, the decrease in NAR was associated with increased foliage in the plants, making the intercepted light lower (Evans and Poorter, 2001).

The NAR is an indirect measure that determines the photosynthetic efficiency of plants. This variable is directly related to the leaf area, layout and age of the leaves and also

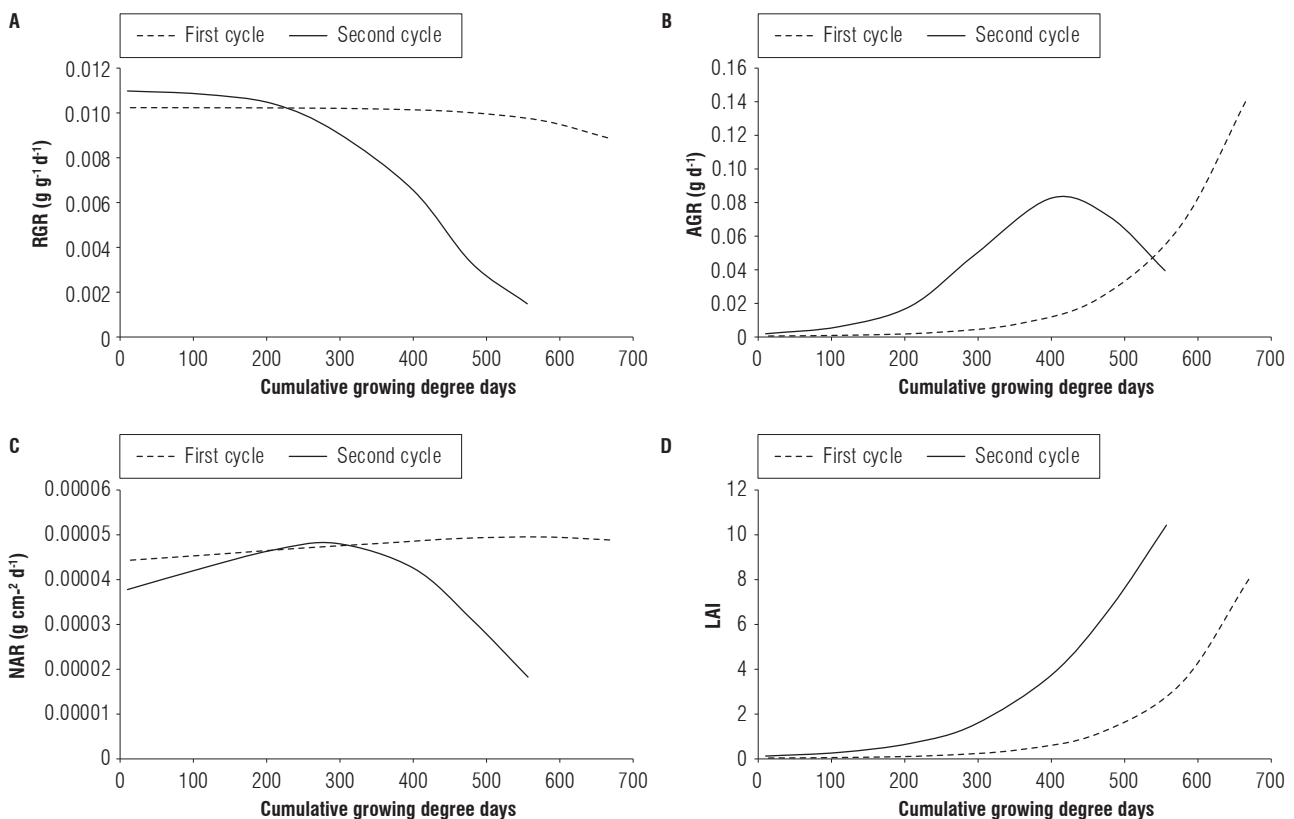


FIGURE 2. Behavior of the A. Relative growth rate (RGR); B. Absolute growth rate (AGR); C. Net assimilation rate (NAR) and D. Leaf area index (LAI), during the growth of lettuce plants for first and second cycle under a plastic cover.

affects the internal metabolism of the plant as a response to external factors through the respiration process (Torres-Moya *et al.*, 2016).

Leaf area index (LAI)

The LAI in the two evaluated cycles had two phases. In the first phase, slow growth was observed until 8 and 15 dat for the first and second cycle respectively; later, accelerated growth was observed until 45 dat, with a maximum value for the first cycle of 8.06 and, for the second one, 10.38, the latter value being the highest LAI (Fig. 2D).

One of the reasons why the first cycle showed a lower accumulation of biomass was probably the low nutrient uptake because of the influence from the roots, which limited the foliar growth during the first days after transplant and affected the production and the growth of new leaves (Martínez and Garcés, 2010). It is known that a plant with a strong root system generates a greater amount of foliage increasing the absorption of solar radiation for synthesizing photoassimilates (Lin *et al.*, 2013). Thus, an increase in the LAI depends on the interception of radiation, temperature, water availability and nutrition (Hernández and Soto, 2012).

Chlorophyll content (CC)

The CC, expressed in SPAD units, for the first cycle in the first 7 dat increased slowly and subsequently decreased again to 15 dat, then presented an accelerated increase, obtaining the maximum value of 27 SPAD at 39 dat; in addition, it can be evidenced that the data during the whole cycle were not stable, but showed constant variations (Fig. 3a). This increase in CC is related to adequate nutrition and with a consequent greater vigor of the plant after the transplant phase.

For the second cycle, in the first days after transplant, the CC decreased slightly, while the plants adapted to the hydroponic system and subsequently presented a rapid increase to 21 dat; thereafter, it remained stable until the end of the cycle (Fig. 3B). The lettuce did not present a high CC because of the light green color that is characteristic of the variety, results agree with Martínez *et al.* (2015), in lettuce variety EZ-1, which showed a low CC because of the presence of carotenoids in the leaves.

The SPAD determines the content of chlorophyll in plants (Barrios *et al.*, 2011). When they have adequate nutrition for N, Mg, Fe and Mn, they have a higher CC since it is related to a higher photosynthetic rate (Coronel *et al.*, 2010). The CC in plants is closely related to the nitrogen content and, therefore, when chlorophyll is at low levels, this may indicate that this nutrient is low in the plant (Castillo and Ligarreto, 2010).

According to Ázcon-Bieto and Talón (2008), nitrogen is important in the formation of chlorophyll since it is part of the cyclic tetrapyrrole ring, linked in the center with a metallic magnesium cation. When there is an absence of this nutrient, apart from having a deficiency, there is a decrease in the content of chlorophyll, affecting the photosynthesis process (Peláez *et al.*, 2010). In leaves, it is very important to quantify the chlorophyll because, when it degrades, the leaves change color from a bright green to brown, or other colors (yellow, purple and orange); these color changes mean a loss of quality in the products (Lin *et al.*, 2013).

Stomatal conductance (SC)

The stomatal conductance in the first cycle, at the time of transplantation, had values lower than $50 \text{ mmol m}^{-2} \text{ s}^{-1}$, then increased slowly until 13 dat; later, the plants accelerated the SC until obtaining the maximum value at 33 dat (382.68

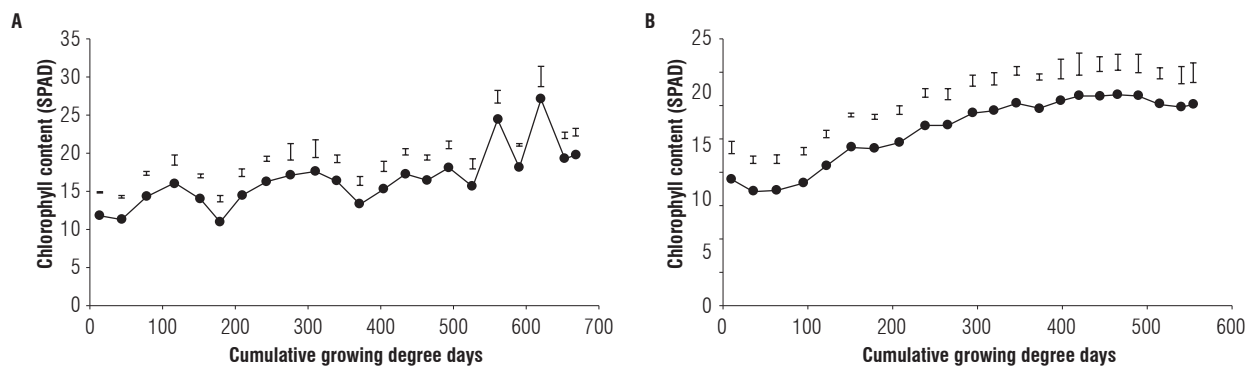


FIGURE 3. Behavior of chlorophyll content (CC) under a plastic cover. A. First cycle; B. Second cycle. The vertical bars indicate the standard error ($n=8$).

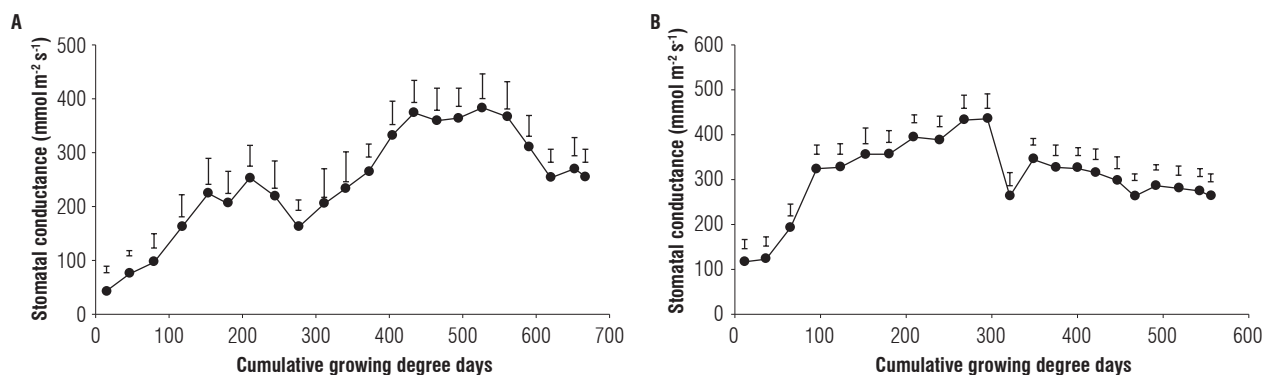


FIGURE 4. Behavior of stomatal conductance (CE) under a plastic cover. A. First cycle; B. Second cycle. The vertical bars indicate the standard error ($n=8$).

$\text{mmol m}^{-2} \text{s}^{-1}$) and finally, the plants decreased the SC as the culture cycle ended (Fig. 4A).

For the second cycle, at the start of transplanting, the SC was low, but it increased slowly until 5 dat, then had an accelerated increase until reaching the maximum value at 21 dat ($436.03 \text{ mmol m}^{-2} \text{s}^{-1}$); afterwards, a rapid decrease was observed until 23 dat, then the SC remained constant until the end of the cycle (Fig. 4B). This indicates that the plants in the first cycle were more stressed due to the problems of pH stabilization of the solution that occurred in this cycle, likewise, these values are higher than reported by Kim *et al.* (2004), who found SC values for lettuce plants ranging from 50 to $130 \text{ mmol m}^{-2} \text{s}^{-1}$ and mention that SC responds directly to the spectral quality of light during growth and that lower SC values do not indicate a greater accumulation of dry mass.

In beet, spinach, tomato and pea plants, when stomata are open, values ranged between $200 \text{ mmol m}^{-2} \text{s}^{-1}$ to $800 \text{ mmol m}^{-2} \text{s}^{-1}$ and when closed, they have low values of $4 \text{ mmol m}^{-2} \text{s}^{-1}$ (Angeles, 2014). When there is a reduction in the SC, it is because of the stomatal closure, which can be caused by several factors, including light, humidity, CO_2 , temperature and air currents (Haworth *et al.*, 2016). Stomatal closure diminishes the CO_2 intake, which inhibits photosynthesis and generates a reduction of photosynthesis (Kawasaki *et al.*, 2015); similarly, abscisic acid regulates SC decreases, causing stomatal closure, when the mesophyll begins to suffer dehydration.

Conclusions

According to the growth of the plants in the NFT hydroponic system, harvest of lettuce plants can occur at 43 dat; in addition, the behavior of the total dry mass was fit to a simple sigmoid growth logistic model. The RGR had its

highest point at the time of planting. The AGR behaved as a sigmoid model and had its maximum value at 43 and 36 dat for the first and second cycles, respectively. The NAR saw its maximum value at 36 dat and began to decrease when plants showed a higher leaf area. The highest LAI was performed in the second cycle because the plants had better development. The CC for this lettuce was low because of the yellow-green pigmentation, characteristic of the variety.

Acknowledgments

The authors would like to thank the Dirección de investigaciones (DIN) of the Universidad Pedagógica y Tecnológica de Colombia for the financing of the project SGI-1578 through the announcement DIN 005-2014 - Proyectos Externos Regionales.

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