# Assessing growth and antioxidant properties of greenhouse-grown lettuces (*Lactuca sativa* L.) under different irrigation and carbon fertilization management

## Evaluación de la producción y propiedades antioxidantes de lechuga (*Lactuca sativa* L.) bajo invernadero en función del manejo del riego y la fertilización carbónica

Idoia Garmendia [1*], Marcelle M. Bettoni [2], Nieves Goicoechea [3]

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### Abstract

Previous studies have demonstrated that moderate water restrictions and enhanced $CO_2$ concentration can maintain or improve yield and accumulation of secondary compounds in lettuce under greenhouse conditions. Therefore, the aim of this study was to evaluate the combination of reduced soil moisture and carbon fertilization in shoot fresh weight (FW) and antioxidant capacity of two Batavia lettuce cultivars (Batabia Rubia Munguia; BRM and Maravilla de Verano; MV). Moderate water restriction treatment was equivalent to 2/3 of the field capacity and elevated $CO_2$ concentration ($ECO_2$) was fixed at ~700 µmol $mol^{-1}$. While $CO_2$ enrichment exerted a positive effect on shoot FW of MV, especially in combination with water restrictions, the yield of the cultivar BRM was not affected by $CO_2$ concentration, nor by irrigation regime. However, antioxidant capacity of BRM plants was increased under $ECO_2$ conditions. These results demonstrate that carbon fertilization and/or moderate water limitations can be strategically used to enhance nutritional value and growth of greenhouse lettuce.



**Keywords**

antioxidant activity • carbon dioxide • *Lactuca sativa* • yield • water restriction • fertilization



1 Universidad de Alicante. Facultad de Ciencias. Departamento Ciencias de la Tierra y del Medio Ambiente. Ctra. San Vicente del Raspeig, s/n. Apdo. Correos 99. E-03080 Alicante. Spain. * idoia.garmendia@ua.es

2 Universidade Federal do Paraná. Setor de Ciências Agrárias Departamento de Fitotecnia e Fitossanitarismo. Rua dos Funcionários. 1540. Juvevê. Curitiba. Brasil.

3 Universidad de Navarra. Facultades de Ciencias y de Farmacia y Nutrición. Departamento de Biología Ambiental. Grupo de Fisiología del Estrés en Plantas (Unidad Asociada al CSIC, EEAD, Zaragoza e ICVV, Logroño). Irunlarrea 1. 31008. Pamplona. Spain.

## Resumen

Estudios previos han demostrado que la moderada reducción del riego y el aumento de la concentración de $CO_2$ pueden mantener o incrementar la producción vegetal e inducir la acumulación de compuestos secundarios en lechuga bajo condiciones de invernadero. Por ello, el objetivo del presente estudio fue evaluar la combinación de la reducción del contenido de humedad del suelo y la fertilización carbónica sobre la materia fresca (MF) de la parte aérea y capacidad antioxidante de dos cultivares de lechuga Batavia (Batavia Rubia Munguia; BRM y Maravilla de Verano; MV). El tratamiento de déficit hídrico moderado fue equivalente a 2/3 de la capacidad de campo y la elevada concentración de $CO_2$ ($ECO_2$) se fijó en ~700 µmol $mol^{-1}$. Mientras que el enriquecimiento de $CO_2$ ejerció un efecto positivo sobre la MF de la parte área de MV, especialmente en combinación con la restricción del riego, la producción del cultivar BRM no se vio afectado ni por la concentración de $CO_2$ ni por el régimen de riego aplicado. Sin embargo, la capacidad antioxidante de las plantas BRM se incrementó bajo condiciones de $ECO_2$. Los resultados demuestran que la fertilización carbónica y/o una moderada limitación hídrica pueden estratégicamente ser utilizados para mejorar el valor nutricional y rendimiento de la lechuga bajo invernadero.



**Palabras clave**

actividad antioxidante • dióxido de carbono • *Lactuca sativa* • rendimiento • restricción del riego • fertilización


## Introduction

Lettuce (*Lactuca sativa* L.) is considered a major food crop within the European Union. It is one of the most popular vegetables given its healthy properties, attributable to the presence of fiber, antioxidant compounds and minerals (12, 16, 18). Batavia (*Lactuca sativa* L. var. *capitata*) is extensively cultivated in greenhouses and highly commercialized in the North of Spain. Batavia Rubia Munguia (BRM) and Maravilla de Verano (MV) are two cultivars of Batavia. BRM develops a round, dense head and has yellow-green leaves, with very ruffled borders and a consistent, crisp texture. MV has rounded and very broad leaves with crunchy texture and red pigmentation it develops a firm head and can be grown all year long, especially in summer due to its resistance to high temperatures.

Cultivation of lettuce requires frequent irrigation for better growth and development because this plant lacks of a deep root system. However, soil moisture ranging from 50 to 75% of field capacity (FC) allow lettuce plants to produce similar biomass than those fully irrigated (20). Moreover, Baslam and Goicoechea (2012) observed that the nutritional quality of BRM and MV had even increased when plants had received 2/3 of FC. When grown with limited irrigation the levels of anthocyanins in the leaves of BRM were higher than in well-watered plants and so were the concentrations of total carotenoids and chlorophylls in leaves of MV.

Regarding the increase of atmospheric $CO_2$ as a consequence of global change and/or horticultural practices, it is well known that it affects plant growth and development. The enhanced $CO_2$ concentration increases the potential net photosynthesis in C3 plants, such as lettuce, and therefore can improve yield over short-term exposures (14). These responses may occur in natural ecosystems, but also can be used to increase the production, the nutritional quality and the accumulation of secondary compounds. Under greenhouse conditions, $CO_2$ fertilization facilitated rapid nursery production of olive trees during the winter season under Mediterranean conditions (5), induced the synthesis of secondary compounds with pharmacological interest in several plant species (17) and induced the accumulation of phenolic compounds in lettuces cv. BRM and MV when plants were not associated with mycorrhizal fungi (2). In fact, Becker and Kläring (2016) observed that $CO_2$ enrichment can result in high yields of red lettuce rich in phenolic compounds.

All these previous findings lead us to hypothesize that the increased levels of some secondary compounds in leaves of BRM and MV cultivated with restricted irrigation or carbon fertilization may enhance their antioxidant properties.

## Objective

The objective was to study whether water deficit and/or carbon fertilization could improve plant growth together with leaf antioxidants in greenhouse grown lettuce.

## Materials and methods

### Plant material and experimental design

Seeds of BRM and MV were surface sterilized by 10% bleach for 10 min and then germinated in a mixture of light peat and sand (1:1, v:v). When seedlings had 2-3 fully developed leaves (three weeks after sowing) 24 plants of each cultivar (BRM and MV) were transplanted to 13 L pots filled with a mixture of vermiculite-siliceous sand-light peat (2.5:2.5:1, v:v:v).

The peat had a pH of 5.2-6.0, 70-150 mg $L^{-1}$ of nitrogen, 80-180 mg $L^{-1}$ of total $P_2O_5$ and 140-220 mg $L^{-1}$ $K_2O$ and it was previously sterilized at 100°C for 1 h on three consecutive days.

During transplant, the plants were transferred to four [$CO_2$] controlled greenhouses located on the Universidad de Navarra campus (42.80 N, 1.66 W; Pamplona, Spain) (two ambient $CO_2$ ($ACO_2$) and two elevated $CO_2$ ($ECO_2$) greenhouses).

The design of the greenhouses was similar to that described by Morales *et al.* (2014). Twelve BRM plants and twelve MV plants were placed in $ACO_2$ greenhouses (six plants from every lettuce cultivar in each $ACO_2$ greenhouse). Twelve BRM plants and twelve MV plants were placed in $ECO_2$ greenhouses (six plants from every lettuce cultivar in each $ECO_2$ greenhouse). In the two $ACO_2$ greenhouses, no $CO_2$ was added and the [$CO_2$] in the atmosphere was approximately 392 μmol $mol^{-1}$. In the other two greenhouses ($ECO_2$), [$CO_2$] was fixed at ~700 μmol $mol^{-1}$ by injecting pure $CO_2$ (purity up to 99.99%) from cylinder-gases (34 L of $CO_2$ per cylinder) through the two inlet fans during the light hours.

The $CO_2$ was provided by Air Liquide (Bilbao, Spain). The [$CO_2$] was continuously monitored using a Guardian Plus gas monitor (Edinburgh Instruments Ltd, Livingston, UK).

Different irrigation regimes were also imposed at transplanting. Six BRM and six MV lettuces cultivated at $ACO_2$ concentration in the air (three plants from each cultivar and $ACO_2$ greenhouse) were always watered at FC and kept as well-watered (WW) treatments. Field capacity was calculated as the maximum water retained by a pot after complete drainage of water excess (900 mL per pot as an average value for all pots). Previous experience (2) demonstrated that WW lettuce plants performed better by dividing this amount of total water into three irrigations per week as follows: 300 mL of Hewitt's nutrient solution with some modifications (1) once a week and 300 mL of distilled water twice a week.

Plants subjected to 2/3 FC received 300 mL of modified Hewitt's solution once a week and 150 mL of distilled water twice a week. In all cases, nutrient solution was alternated with distilled water in order to avoid salt deposition. As results obtained by Baslam and Goicoechea (2012) showed that the leaf water content in WW lettuce plants was at least 90% after receiving the abovementioned nutrient solution and water supplies for 7 weeks, the irrigation regimes in the present study were kept through the whole experiment.

**Total antioxidant capacity of leaves**

Leaf extracts were obtained as described by Chapuis-Lardy *et al.* (2002) with some modifications. Samples (0.5 g of FW) were pulverized in liquid nitrogen, mixed with 20 mL of 80% methanol, and homogenized at room temperature for 1 min. After filtration, 0.5 mL of each sample was mixed with 10 mL of distilled water.

The total antioxidant capacity was evaluated by applying the free α, α-Diphenil-β-picrylhydrazyl radical scavenging activity ($DPPH^•$ assay).

The free radical scavenging activity using the free radical $DPPH^•$ (6) was evaluated by measuring the variation in absorbance at 515 nm after 30 min of reaction in parafilm-sealed glass cuvettes (to avoid methanol evaporation) at 25°C (9).

The reaction was started by adding 20 μL of the corresponding sample to the cuvette containing 80 μM (methanol solution) (980 μL) of the free radical ($DPPH^•$) (11).

The final volume of the assay was 1 mL. Reaction was followed with a spectrophotometer (Jasco V-630, Analytical Instruments, Easton, MD, USA). The trapping potential for DPPH radicals scavenging activity in the leaf extracts was calculated as the percentage of inhibition (*I* %) against blank:

$$I\ \% = (A\ \text{blank} - A\ \text{sample}) * 100 / A\ \text{blank}$$

where:

*A* blank = the absorbance of the control reaction (containing all reagents except the test compound)

*A* sample = the absorbance of the test compound.

**Statistical analysis**

Within each lettuce cultivar, data were subjected to a two-factor ANOVA (factorial 2 × 2) (IBM SPSS v. 24).

The variance was related to the main treatments (atmospheric $CO_2$ concentration, $CO_2$ and water regime, W) and to the interaction between both parameters ($CO_2$ × W). Means ± standard errors (SE) were calculated and, when the F ratio was significant ($p \leq 0.05$), a Duncan Multiple Range Test was applied. Tests results were always considered significant at $p \leq 0.05$.

## Results

Although the shoot FW of the cultivar BRM tended to be higher when plants were cultivated under $ECO_2$ (table 1), the final size achieved by the lettuces was not significantly affected by either the concentration of $CO_2$ or the irrigation regime (table 1). In contrast, the antioxidant capacity measured in leaves of BRM was significantly enhanced when plants were exposed to $ECO_2$ ($CO_2$, $p \leq 0.01$). The interaction between $CO_2$ and water supply was not significant for either growth or antioxidant properties in this cultivar.

Contrary to BRM, growth of MV was clearly increased under $ECO_2$ (table 2, page 92). Moreover, such enhancement was reinforced when $ECO_2$ interacted with restricted irrigation ($CO_2 \times W$, $p \leq 0.05$), so that the highest shoot FW (67.33 g plant$^{-1}$) was achieved by plants cultivated under $ECO_2$ with a water supply of 2/3 FC. In this red-leaf cultivar, the antioxidant capacity measured in leaves was not affected by the irrigation regime, the concentration of $CO_2$ in the air or the interaction between both environmental factors (table 2, page 92).

**Table 1** Shoot fresh weight (FW) (g plant$^{-1}$) and trapping potential for DPPH radicals scavenging activity (*I* %) in Batavia Rubia Munguia (BRM) cultivated at either ambient (~370 µmol mol$^{-1}$) ($ACO_2$) or elevated ($ECO_2$) (~700 µmol mol$^{-1}$) $CO_2$ concentration in the air, and grown with optimal (FC, field capacity) or restricted (2/3 FC) water supply.

**Tabla 1** Materia fresca (FW) de la parte aérea (g planta$^{-1}$) y capacidad para eliminar radicales DPPH (*I* %) en Batavia Rubia Munguia (BRM) cultivada tanto en concentraciones de $CO_2$ ambiente (~370 µmol mol$^{-1}$) ($ACO_2$) como en elevadas ($ECO_2$) (~700 µmol mol$^{-1}$), y crecidas bajo riego óptimo (FC, capacidad de campo) o bajo restricción (2/3 FC).

| Treatments | | Shoot FW (g plant$^{-1}$) | *I* % |
|---|---|---|---|
| $ACO_2$ | FC | 47.12 ± 3.95 | 68.12 ± 1.59 |
| | 2/3 FC | 45.96 ± 4.96 | 74.49 ± 1.92 |
| $ECO_2$ | FC | 50.48 ± 2.49 | 78.17 ± 2.66 |
| | 2/3 FC | 56.92 ± 3.61 | 77.31 ± 1.55 |
| ***Main effects*** | | | |
| $CO_2$ | $ACO_2$ | 46.75 ± 2.93 | 71.02 ± 1.54 b |
| | $ECO_2$ | 53.41 ± 2.25 | 77.74 ± 1.47 a |
| Water supply | FC | 48.95 ± 2.27 | 73.15 ± 2.12 |
| | 2/3 FC | 51.44 ± 3.39 | 76.03 ± 1.23 |
| $CO_2$ | | NS | ** |
| Water supply (W) | | NS | NS |
| $CO_2 \times W$ | | NS | NS |

Values are means (n = 6) ± S.E. Within each column, data followed by the same letter indicate that values did not differ significantly ($p \leq 0.05$). ANOVA: NS, not significant; **, significant at $p \leq 0.01$.

Los valores corresponden a medias muestrales (n = 6) ± S.E. Dentro de cada columna, el valor acompañado por la misma letra indica que las medias no difieren significativamente ($p \leq 0,05$). ANOVA: NS, no significativo; **, significativo a $p \leq 0,01$.

**Table 2** Shoot fresh weight (FW) (g plant$^{-1}$) and trapping potential for DPPH radicals scavenging activity (*I* %) in Maravilla de Verano (MV) cultivated at either ambient (~370 µmol mol$^{-1}$) ($ACO_2$) or elevated ($ECO_2$) (~700 µmol mol$^{-1}$) $CO_2$ concentration in the air, and grown with optimal (FC, field capacity) or restricted (2/3 FC) water supply.

**Table 2** Materia fresca (FW) de la parte aérea (g planta$^{-1}$) y capacidad para eliminar radicales DPPH (*I* %) en Maravilla de Verano (MV) cultivada tanto en concentraciones de $CO_2$ ambiente (~370 µmol mol$^{-1}$) ($ACO_2$) como en elevadas ($ECO_2$) (~700 µmol mol$^{-1}$), y crecidas bajo riego óptimo (FC, capacidad de campo) o bajo restricción (2/3 FC).

| Treatments | | Shoot FW (g plant$^{-1}$) | *I* % |
|---|---|---|---|
| $ACO_2$ | FC | 55.90 ± 3.66 bc | 70.27 ± 4.19 |
| | 2/3 FC | 47.28 ± 2.67 c | 75.32 ± 3.74 |
| $ECO_2$ | FC | 60.27 ± 2.34 ab | 73.07 ± 3.78 |
| | 2/3 FC | 67.33 ± 4.84 a | 74.66 ± 2.23 |
| **Main effects** | | | |
| $CO_2$ | $ACO_2$ | 51.59 ± 2.52 b | 72.79 ± 2.79 |
| | $ECO_2$ | 64.00 ± 2.80 a | 73.86 ± 2.11 |
| Water supply | FC | 58.08 ± 2.17 | 71.67 ± 2.72 |
| | 2/3 FC | 57.51 ± 4.06 | 74.99 ± 2.08 |
| $CO_2$ | | ** | NS |
| Water supply (W) | | NS | NS |
| $CO_2$ × W | | * | NS |

Values are means (n = 6) ± S.E. Within each column, data followed by the same letter indicate that values did not differ significantly ($p \leq 0.05$). ANOVA: NS, not significant; *, significant at $p \leq 0.05$; **, significant at $p \leq 0.01$.

Los valores corresponden a medias muestrales (n = 6) ± S.E. Dentro de cada columna, el valor acompañado por la misma letra indica que las medias no difieren significativamente ($p \leq 0,05$). ANOVA: NS, no significativo; *, significativo a $p \leq 0,05$; **, significativo a $p \leq 0,01$.

## Discussion

The statistical study of the main effects of $CO_2$ and water supply demonstrated that moderate water restriction (2/3 FC) did not reduce yield in any of the two cultivars of lettuce (table 1, page 91 and table 2), despite the high sensitiveness of plant growth to water deficit.

Enhanced $CO_2$ concentration in the air increases the potential net photosynthesis in C3 plants (8), which results in improved plant growth. However, when the synthesis of carbohydrates in plants exposed to $ECO_2$ exceeds the capacity to produce new sinks, their photosynthetic rate declines as a consequence of a product feedback inhibition (19).

In this study, BRM and MV plants were harvested when their vegetative development had not still finished, which means that the youngest leaves could act as sink organs. This fact may explain why $CO_2$ enrichment exerted a positive effect on shoot growth, being this effect more evident in MV (table 2) than in BRM (table 1, page 91).

The antioxidant capacity measured in leaf extracts from the green-leaf BRM was similar to that found in the red-leaf MV (table 1, page 91 and table 2), which contrasts with the significantly higher DPPH scavenging activity found by Llorach *et al.* (2008) in red lettuces in comparison with that of green varieties. According to Baslam *et al.* (2012), leaves of MV have more anthocyanins than those of BRM when plants are fully irrigated and cultivated at $ACO_2$, but the amount of total phenolic compounds is higher in leaves of BRM than in leaves of MV. This suggests that the antioxidant capacity in the red-leaf MV may be mainly due to the anthocyanins (15) and the antioxidant properties of the green-leaf BRM may be related to total phenolics (10).

Baslam *et al.* (2012) also observed that the concentrations of anthocyanins significantly increased in leaves of BRM exposed to $ECO_2$, which could explain the enhancement of the antioxidant capacity observed in this cultivar fertilized with $CO_2$ (table 1, page 91). At $ACO_2$, moderate water restriction (2/3 FC) also induced the accumulation of anthocyanins in leaves of BRM (2), which may explain the higher trapping potential for DPPH radicals scavenging activity in BRM receiving irrigation equivalent to 2/3 FC (74.49%) than in plants grown with full irrigation (68.12 %) (table 1, page 91).

However, no additive effect between restricted irrigation (2/3 FC) and $ECO_2$ was observed for improving the antioxidant activity in neither of the two cultivars (table 1, page 91 and table 2, page 92). This lack of interaction between $CO_2$ and water restriction for increasing the antioxidant properties may be due to the preferential use of the photoassimilates to improve growth in detriment to the synthesis and accumulation of secondary compounds in plants cultivated with restricted water supply (2/3 FC) under $ECO_2$.

In BRM (table 1, page 91), while atmospheric $CO_2$ fertilization increased shoot FW by 7% in fully irrigated plants (from 47.12 to 50.48 g plant$^{-1}$), the increase in plants grown with water restriction (2/3 FC) was 24% (from 45.96 to 56.92 g plant$^{-1}$).

In MV (table 2, page 92), $ECO_2$ enhanced shoot FW by 8% in plants with full irrigation (from 55.90 to 60.27 g plant$^{-1}$) and by 42% (from 47.28 to 67.33 g plant$^{-1}$) in plants receiving 2/3 FC.

## Conclusions

Moderate limitation of water supply may allow the obtention of greenhouse-grown lettuces without significant decreases in the final plant size. Carbon fertilization can enhance yield and/or the antioxidant properties of greenhouse-grown lettuces, but results are highly dependent on the variety or cultivar evaluated.

Only in the red-leaf cultivar of lettuce, MV, plant growth (but not the antioxidant activity) was improved by the simultaneous application of restricted irrigation and $CO_2$ enrichment.

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