UNIVERSIDAD
DE ALMERÍA

**INTERNATIONAL SCHOOL OF DOCTORATE**

Greenhouse Technology and Industrial and Environmental Engineering Ph.D. Program

# *Increase in production and phytochemicals: use of silicon, ammonium, artificial light and Matlab.*

*Aumento de la producción y de los fitoquímicos: uso del silicio, amonio, luz artificial y Matlab.*

**Ph.D. student:**
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## AUTHORIZATION OF THE DIRECTOR / S OF THE Ph.D. THESIS FOR SUBMISSION

Dr. Miguel Urrestarazu Gavilán and Dr. Jose Luis Guil Guerrero as Directors of the Ph.D. Thesis entitled “Increase in production and phytochemicals: use of silicon, ammonium, artificial light and Matlab." carried out by Francisca Carrillo Ferrón in the Department of Agronomy, authorizing its presentation for processing since it meets the necessary conditions for its defense.

What we signed, to comply with Royal Decree 99/2011, 1393/2007, 56/2005 and 778/98, in Almería on June, 2021.

ESCUELA INTERNACIONAL DE DOCTORADO
UNIVERSIDAD DE ALMERÍA

Dr. Miguel Urrestarazu Gavilán

Dr. Jose Luis Guil Guerrero

# Aknowledgements

To my parents, my teachers and my classmates, for shaking my hand, in accepted and rejected.

Index

# Resumen

La finalidad de este trabajo ha sido la obtención de una mejora en la calidad de la producción en los cultivos sin suelo, pudiendo aplicarse además en ciertos casos a los cultivos en suelo. Esto se debe al manejo de las nuevas tecnologías como son el uso de cámaras termográficas y la aplicación de algoritmos en Matlab.

Para ello, se ha realizado un control de la disolución nutritiva y de las técnicas de iluminación artificial, teniendo en cuenta la preservación del medio ambiente.

La correcta gestión del agua y de la fertirrigación, junto con el empleo de diferentes elementos químicos en las soluciones nutritivas como son el silicio y el amonio, resultan esenciales a la hora de alcanzar un aumento en la producción, junto con el empleo de diversos sustratos como son la fibra de coco y la arena silícea, para favorecer la concentración de fitoquímicos beneficiosos para la salud humana.

Como resultado, se obtienen las concentraciones y condiciones idóneas para el aumento del rendimiento y de la potenciación de compuestos químicos en diferentes cultivos como son pepino, melón, pimiento, lechuga y arándano, pudiendo extrapolarse a otras muchas especies.

# Abstract

The aim of this work has been to obtain an improvement in the quality of production in soilless crops, which can also be applied in certain cases to soil crops. This is due to the use of new technologies such as the use of thermographic cameras and the application of algorithms in Matlab.

To this end, a control of nutrient solution and artificial lighting techniques has been carried out, taking into account the preservation of the environment.

The correct management of water and fertigation, together with the use of different chemical elements in the nutrient solutions such as silicon and ammonium, are essential to achieve an increase in production, together with the use of different substrates such as coconut fibre and siliceous sand, to favour the concentration of phytochemicals that are beneficial to human health.

As a result, the ideal concentrations and conditions are obtained for increasing yields and boosting chemical compounds in different crops such as cucumber, melon, pepper, lettuce and blueberry, and can be extrapolated to many other species.

# I Introduction

## Introducción

Se estima que, en las próximas décadas, la población mundial crecerá alrededor de un 33%, lo que supondrá que para 2050, en el mundo convivirán unos 10.000 millones de personas (FAO, 2020).
La agricultura 4.0 pretende producir de manera distinta a lo que se viene haciendo de forma convencional, utilizando técnicas alternativas como las plantaciones de cultivo sin suelo (De Clerq et al., 2018).

En la actualidad además de producir con suficiente cantidad para alimentar a la población mundial, se pretende potenciar el valor nutricional y contenido en fitoquímicos beneficiosos para la salud humana de los productos cultivados de una forma sostenible con la mayor eficacia y eficiencia posible. Por tanto también se quiere cultivar de una forma sostenible utilizando 1) el menor número posible de recursos generales incluyendo el espacio físico, con la mínima injerencia en el medio ambiente, 2) incorporando el concepto de economía circular (Arroyo-Morocho, 2018) y 3) con el mayor respeto a las consideraciones sociales (Chaves-Ávila et al., 2018).

Por otro lado, un manejo eficiente de la nutrición mineral y los parámetros de fertirrigación en los cultivos garantiza un buen crecimiento y una producción de alta calidad (Vargas y Bryla, 2015).

En la mayoría del terreno mundial destinado a la agricultura la obtención de buenos rendimientos, así como también el poder cultivar una amplia variedad de especies, cada vez está teniendo más restricciones debido a la salinización de los suelos. Se estima que sobre 780 millones de hectáreas en el planeta están afectadas por sales, de estas 400 millones lo son por problemas de salinidad y 519 millones por condiciones asociadas a sodicidad (FAO, 2017).

Uno de los factores principales que influyen en el desarrollo y la producción de los cultivos es el sustrato, y la solución de nutrientes, que puede interferir con la disponibilidad de nutrientes (Urrestarazu, 2015).

El silicio como nutriente es ampliamente tratado en multitud de bibliografia (Ferrón-Carrillo et al., 2019; Esmaili et al., 2021), sin embargo su papel en múltiples procesos que afecta a la producción vegetal aun está lejos de ser totalmente aclarado. En la actualidad, la información existente sobre los beneficios del uso de diferentes elementos en la solución nutritiva como el silicio (Si) en agricultura es cada vez mayor (Urrestarazu et al., 2016). Especialmente, su significativa utilidad a la hora de paliar situaciones de estrés (Xia et al., 2018). También su contribución en el metabolismo para la formación de tricomas y fitolitos como defensa y fortalecimiento (Ferrón-Carrillo et al., 2019). Además de suponer una ayuda en el retraso de la aparición de nuevas sequías consecuencia de un creciente calentamiento global.

Por ejemplo, la aplicación de Si aumenta significativamente la concentración de calcio en brotes de plantas de pepino expuestas a la salinidad, -habida cuenta de que la presencia de calcio en el cultivo favorece la defensa del mismo-, mientras que no tiene efecto en la concentración de la calcio de brotes de plantas cultivadas bajo condiciones no salinas (Khoshgoftarmanesh et al., 2014).

El Si, además, es un elemento fundamental como nutriente para los arándanos (Morikawa et al., 2004). Los efectos negativos del exceso de fertilización nitrogenada pueden ser mitigados por la rigidez conferida por el aporte de Si (Guntzer et al., 2012), por lo que permite utilizar menores cantidades de fertilizante y por tanto menores emisiones de nitrógeno al medio ambiente.

Aun podemos encontrar lagunas de conocimiento en las funciones del Si en las formaciones epicuticulares tanto del vástago como de fruto, así como en su papel directo en las repercuciones sobre la fitoquímica de plantas hortícolas.

Desde la década de los 70s se han formulado infinidad de soluciones nutritivas para distintas situaciones de sustratos y plantas hortícolas. Muchas de ellas cuentan con la presencia de nitrógeno en forma amónica (Cox y Reinsenaure, 1973; Clarkson et al. 1986; Forde y Clarkon, 1999). Entre las mas antiguas que recogen el amonio en su destacamos las de Blacquiere et al. (1988) encontrando rendimientos incrementados de un 40 a un 70%.

Otro de los aspectos de alto grado de implicación e interrelación con el fertirriego en horticultura es la influencia de la intensidad y calidad de la luz tanto natural como artificial con la composición fitoquímica de los productos hortícolas. El uso de luz LED con espectro predominante del rojo para impulsar la fotosíntesis ha sido ampliamente aceptado por dos razones principales. Primero, las curvas de McCree (Sager y McFarlane, 1997) indican que las longitudes de onda del rojo (600 a 700 nm) son absorbidas de manera eficiente por los pigmentos vegetales; en segundo lugar, los primeros LED eran de color rojo con la emisión más eficiente a 660 nm, cerca de un espectro de absorción de las clorofilas.

Este tipo de iluminación, se puede utilizar para diferentes especies vegetales, las cuales pueden variar en su contenido en carotenoides por su importancia, como son los microgreens. Por ejemplo, (Paradiso et al., 2018) indicaron que *Lactuca sativa* L. cv. contiene concentraciones significativas de carotenoides en la etapa microverde. Además, los microgreens de Brassicaceae, como el brócoli, el repollo y el rábano, contienen altas cantidades de glicosilonatos, que se informan como compuestos antitumorales (Kopsell et al., 2013). Sin embargo, existe evidencia de que los nitratos reaccionan con otras aminas secundarias para producir nitrosaminas, lo que induce cáncer gastrointestinal (Hord et al., 2009).

En la moderna horticultura y su fertirrigación se destaca la monitorización mediante sensores de todo tipo: humedad, temperatura, conductividad eléctrica, pH, nitratos y potasio. Esta aplicación de sensores facilita por tanto la elección del sistema de distribución de la aplicación de nutrientes en el sustrato para un mejor desarrollo de las raíces. La monitorización ambiental tiene muchas aplicaciones con el objetivo de mejorar la sostenibilidad de los recursos naturales (Novas et al., 2017).

Otro aspecto novedoso en el control del fertirriego procede del uso de las termografías. Se puede realizar una investigación más profunda de la disponibilidad de agua y nutrientes, lo que resulta en un mayor crecimiento de la longitud y volumen de las raíces (Robinson, 1994). Es de gran importancia investigar el potencial de la termografía infrarroja como una herramienta para monitorear las raíces en unidades de cultivo sin suelo, tanto para la detección de enfermedades como para conocer el vigor del cultivo (Fernández Bregón et al., 2013).

Una de sus múltiples aplicaciones en agronomía es el manejo de sistemas de fertirrigación para control de la distribución de las raíces en el sustrato (Fernández-Bregón et al., 2013), o análisis de estrés hídrico (Urrestarazu, 2013), siendo de gran ayuda para estudiar la eficacia del uso de los recursos hídricos para aplicaciones en los cultivos (Antonucci et al., 2011) y en especial plantas en macetas en un cultivo sin suelo (Urrestarazu, 2013).

## Introduction

It is estimated that, in the coming decades, the world's population will grow by around 33%, which means that by 2050, the world will be home to around 10 billion people (FAO, 2020). Agriculture 4.0 aims to produce in a different way to what has been done conventionally, using alternative techniques such as soil-less plantations (De Clerq et al., 2018).

Nowadays, in addition to producing sufficient quantities to feed the world's population, the aim is to enhance the nutritional value and phytochemical content beneficial to human health of the products grown in a sustainable way as effectively and efficiently as possible. Therefore, the aim is also to grow crops in a sustainable way using 1) as few overall resources as possible, including physical space, with the minimum interference with the environment, 2) incorporating the concept of circular economy (Arroyo-Morocho, 2018) and 3) with the greatest respect for social considerations (Chaves-Ávila et al., 2018).

In the other hand, efficient management of mineral nutrition and fertigation parameters in crops ensures good growth and high quality production (Vargas and Bryla, 2015).

In most of the world's agricultural land, obtaining good yields, as well as being able to grow a wide variety of crops, is increasingly constrained by soil salinisation. It is estimated that over 780 million hectares on the planet are affected by salts, of which 400 million hectares are affected by salinity problems and 519 million hectares by conditions associated with sodicity (FAO, 2017).

One of the main factors influencing crop development and production is the substrate, and the nutrient solution, which can interfere with nutrient availability (Urrestarazu, 2015). Silicon as a nutrient is widely discussed in a multitude of literature (Ferrón-Carrillo et al., 2019;

Esmaili et al., 2021), however its role in multiple processes affecting plant production is still far from being fully elucidated. Currently, there is increasing information on the benefits of the use of different elements in the nutrient solution such as silicon (Si) in agriculture (Urrestarazu et al., 2016). Especially, its significant usefulness in alleviating stress situations (Xia et al., 2018). Also its contribution in metabolism for the formation of trichomes and phytoliths as a defence and strengthening (Ferrón-Carrillo et al., 2019). It also helps to delay the onset of new droughts as a consequence of increasing global warming.

For example, the application of Si significantly increases the concentration of calcium in shoots of cucumber plants exposed to salinity, given that the presence of calcium in the crop favours its defence, while it has no effect on the concentration of calcium in shoots of plants grown under non-saline conditions (Khoshgoftarmanesh et al., 2014).

Si is also a key nutrient for blueberries (Morikawa et al., 2004). The negative effects of excess nitrogen fertilisation can be mitigated by the stiffness conferred by the Si input (Guntzer et al., 2012), thus allowing lower amounts of fertiliser to be used and thus lower nitrogen emissions to the environment.

There are still gaps in knowledge on the role of Si in epicuticular formations of both stem and fruit, as well as its direct role in the impact on the phytochemistry of horticultural plants.

Since the 1970s, a multitude of nutrient solutions have been formulated for different substrate situations and horticultural plants. Many of them include nitrogen in the form of ammonium (Cox and Reinsenaure, 1973; Clarkson et al. 1986; Forde and Clarkon, 1999). Among the older ones that include ammonium in their ammonium content, Blacquiere et al. (1988) found increased yields of 40 to 70%.

Another aspect with a high degree of involvement and interrelation with fertigation in horticulture is the influence of the intensity and quality of both natural and artificial light on the phytochemical composition of horticultural products. The use of red-dominant LED light to drive photosynthesis has been widely accepted for two main reasons. First, McCree curves (Sager and McFarlane, 1997) indicate that red wavelengths (600 to 700 nm) are efficiently absorbed by plant pigments; secondly, early LEDs were red in colour with the most efficient emission at 660 nm, close to the absorption spectrum of chlorophylls.

This type of illumination can be used for different plant species, which may vary in their carotenoid content because of their importance, such as microgreens. For example, (Paradiso et al., 2018) indicated that *Lactuca sativa* L. cv. contains significant concentrations of carotenoids in the microgreen stage. In addition, Brassicaceae microgreens, such as broccoli, cabbage and radish, contain high amounts of glycosylonates, which are reported to be anti-tumour compounds (Kopsell et al., 2013). However, there is evidence that nitrates react with other secondary amines to produce nitrosamines, which induce gastrointestinal cancer (Hord et al., 2009).

In modern horticulture and its fertigation, monitoring by sensors of all kinds - humidity, temperature, electrical conductivity, pH, nitrates and potassium - is prominent. This application of sensors thus facilitates the choice of the distribution system for the application of nutrients in the substrate for better root development. Environmental monitoring has many applications with the aim of improving the sustainability of natural resources (Novas et al., 2017).

Another novel aspect in fertigation monitoring comes from the use of thermography. A deeper investigation of water and nutrient availability can be carried out, resulting in increased root length and volume growth (Robinson, 1994). It is of great importance to investigate the potential of infrared thermography as a tool to monitor roots in soilless cultivation units, both for disease detection and crop vigour (Fernández Bregón et al., 2013).

One of its multiple applications in agronomy is the management of fertigation systems to control root distribution in the substrate (Fernández-Bregón et al., 2013), or water stress analysis (Urrestarazu, 2013), being of great help to study the efficiency of the use of water resources for crop applications (Antonucci et al., 2011) and especially potted plants in soilless cultivation (Urrestarazu, 2013).

# II Objectives

## General objectives

The general objective of this thesis is to propose improvements in fertigation in soilless cultivation systems that increase the quality of production with the use of modern technologies from thermography to the composition of the nutrient solution itself and the use of modern lighting techniques, all with the strictest care for the environment.

The specific objectives are detailed in each chapter.

## Specific objectives

### Objective 1.

Efficient management of fertigation in soilless cultivation. It is intended to achieve an optimization in the use of water and nutrients. For this, different elements are used in the nutritional solutions evaluated: Silicon and Ammonium. It will be studied in different protected horticultural crops such as cucumber, melon and pepper.

### Objective 2.

Enhancement of the production of phytochemicals beneficial to human health. Artificial lighting techniques are used in soilless cultivation to measure concentrations of nitrates and carotenoids in lettuce varieties.

The evaluation of different concentrations of silicon is carried out, in addition to the use of several substrates such as coconut fibre and silicon sand for the production of phenolsic acids and antioxidant activity in blueberries.

Objective 3.

Application of automation in crop control. For this, we resort to the use of thermography and the implementation of algorithms through Matlab.

# III Chapter I: Research papers

# 1. Effects of Si in nutrient solution on leaf cuticles

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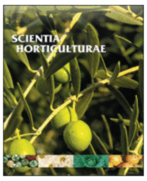

# Effects of Si in nutrient solution on leaf cuticles

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## ABSTRACT

The benefits of silicon for different plant species have been described in many studies in both soilless and traditional soil culture systems. The aim of this work was to quantify the effect of Si on leaf cuticles under different fertigation regimes and the relationship of this effect to water, potassium and nitrate absorption, vegetative growth and plant protection. Cucumber, melon and pepper plants were transplanted into coconut fiber containers with Si in the nutrient solution at 0.6 mM (+Si) and without Si (-Si) under optimal fertigation (OF) and moderate deficit fertigation (DF) conditions. Absorption of water, nitrate and potassium, vegetative growth, leaf firmness, loss of water through cuticle transpiration, cuticle thickness, number of trichomes and Si content in the epidermis and trichomes were measured. Trichome numbers, cuticle thickness and Si content were examined using light and SEM microscopes equipped for X-ray microanalysis. Resistance to two pathogens, *Botrytis cinerea* and *Erysiphe cichoracearum*, was also measured. The results show a loss of growth in the three cv. under DF that was alleviated when Si was supplied in the nutrient solution. +Si significantly improved water absorption and decreased leaf loss, which may explain the improvement shown in the growth parameters. +Si showed clear and significant growth increases in both the epidermis and the cuticle, which could justify both the observed greater resistance to diseases and the lower rates of water loss from the leaves. The three cv. showed high concentrations of Si in the trichomes, even under -Si treatments with a mean Si concentration lower than that in the +Si treatments.

## 1. Introduction

The benefits of silicon for different plant species grown in hydroponics and in traditional soil have been described in many studies (Samuels et al., 1993; Datnoff et al., 2001; Hernandez-Apaolaza, 2014; Savvas and Ntatsi, 2015; Mantovani et al., 2018). These benefits are especially evident when plants grow in adverse environments under both biotic and abiotic stresses (Cooke and Leishman, 2011). While the mechanisms by which plants use silicon in their defense are far from fully understood, the beneficial effects of silicon are considered to act both actively and passively through cuticle thickening (Van Bockhaven et al., 2013), although the exact mechanism(s) by which silicon modulates plant physiology through the potentiation of host defense mechanisms still needs further investigation at the genomic, metabolomic, and proteomic levels (Debona et al., 2017). When silicon precipitates in its different forms in the epidermis of plants, it influences their physical functions, such as transpiration and mechanical resistance (Cooke and Leishman, 2011; Ma and Takahashi, 2002; Romero-Aranda et al., 2006; Tafolla-Arellano et al., 2018).

Silicon allows improved growth under saline conditions without negative repercussions for the plants, since the silicon also decreases sodium absorption when sodium is in excess (Ahmad and Zaheer, 1992; Ma et al., 2001; Saqib et al., 2008).

Improved plant development has also been reported in herbaceous (Pozo et al., 2015) and woody (Gallegos-Cedillo et al., 2018) plants, as well as improved water (Ma et al., 2001), K (Mali and Aery, 2008a) and nitrogen (Mali and Aery, 2008b) absorption both in hydroponics and in soil.

On the other hand, it is well known that the presence of Si in the leaves increases resistance to hydric stress and salinity, reducing the membrane permeability and transpiration through the cuticle, and consequently improves the hydric balance of the plant (Romero-Aranda et al., 2006). The negative effects of an excess of nitrogen fertilization, which allows fast growth and consequently weaker tissues that are susceptible to attack by plagues and diseases, can be mitigated the rigidity conferred by silicon and the reduction in N absorption (Guntzer et al., 2012).

The physical strength of the leaf resulting from Si accumulation in

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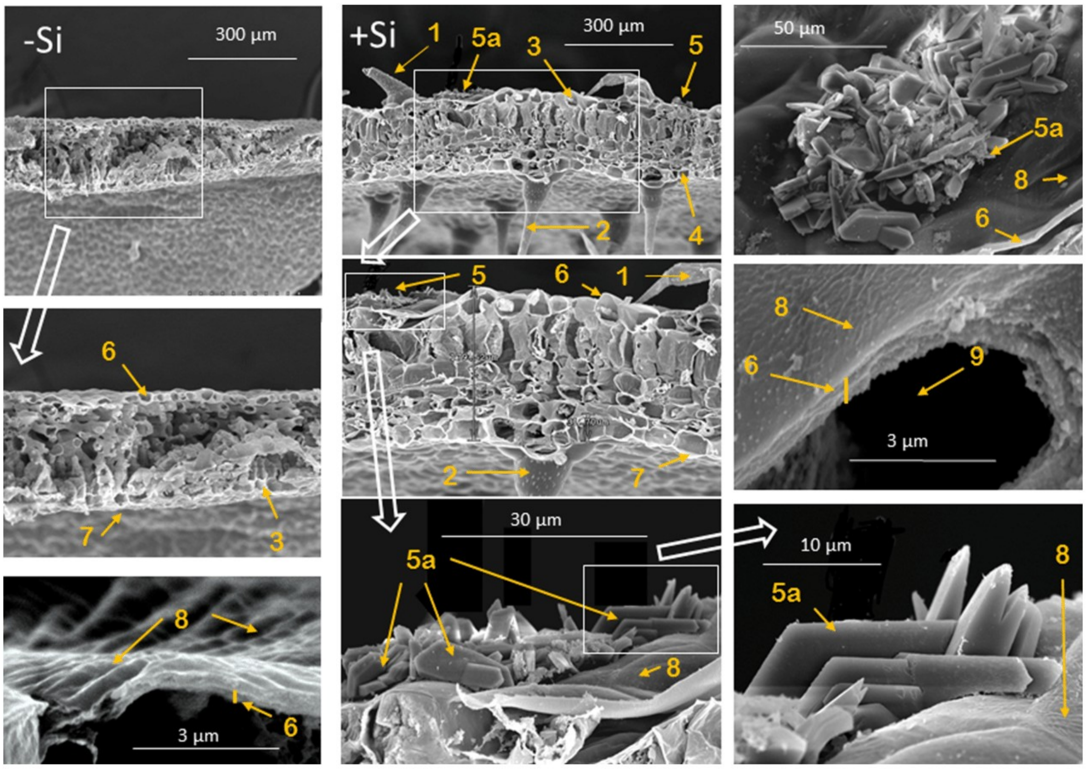

**Fig. 1.** Scanning electron microscopy images of pepper leaf cross-sections treated with Si in nutrient solution (+Si) (second and third columns) versus a control plant (-Si) (first column). 1 and 2 are trichomes on adaxial and abaxial surfaces. 3 and 4 are adaxial and abaxial epidermal cells, respectively. 5 is the presence of epicuticular crystals and phytoliths on the adaxial surface of leaves, and 5a indicates the same crystal group under different views. 6 and 7 are adaxial and abaxial cuticles, respectively. 8 show the wrinkled epicuticular structure on the adaxial surface and upper epidermal wall. 9 is a space epidermal cell.

the cuticle can explain the mechanism of protection against certain bacterial (Dannon and Wydra, 2004) or fungal diseases well known in rice (Zhang et al., 2013; Schurt et al., 2014) and vegetables (Pozo et al., 2015).

A wide variety of methods are used to apply commercial silicon-based products, including sporadic and continuous applications through fertigation. Although ranges higher than 2 mM can be found, Si in the nutrient solution is usually used at a dose of 0.6–1.5 mM. Authors such as Sonneveld and Straver (1994), recommend 0.5 mM of Si for lettuce and 0.75 for cucurbits such as cucumber and melon, and do not include Si in the nutritive solution for Solanaceae such as tomato and pepper; while Pozo et al. (2015) have found significant benefits and increased leaf and stem cuticle thickness under 0.6 mM in lettuce, tomato, pepper, melon and cucumber. In soilless culture with increasing doses in blueberry cultivation, positive effects have also been found under up to 1.2 mM Gallegos-Cedillo et al. (2018).

The objective of this work was to quantify the effects of Si on the cuticles of cucumber, melon and pepper plants under optimal and deficient fertigation and its relationship with 1) the absorption of water, potassium and nitrate, 2) vegetative development, 3) leaf firmness, and 4) protection against powdery mildew and botrytis.

## 2. Materials and methods

### 2.1. Plant growth conditions and fertigation treatments

The experiment was carried out at the University of Almeria (Spain) in an "almeria"-type plastic greenhouse. Seedlings of cucumber (*Cucumis sativus* L. cv. 21 PE 512), melon (*C. melo* L. cv. Nerval) and pepper (*Capsicum annuum* L. cv. Glabriusculum) were transplanted on 15 February 2018 into individual 2.5 L potscontainers. The substrate used was commercial coconut fiber, the physicochemical characteristics of which are described in Pozo et al. (2014). Two treatments were used to apply fertigation: an optimum fertigation volume (FO) and a deficit fertigation volume (FD). FO fertigation management was carried out following the criteria of Urrestarazu, 2015, where each new fertigation application was carried out when the water in the growing unit had reached 10 % of the easily available water following the recommendation of Rodríguez et al. (2014). For each treatment, four repetitions were made in which the drainage from the pot was collected. Fertigation and drainage were averaged daily for 75 days. Volume, pH, electrical conductivity (EC), and concentration of nitrates and potassium were measured and their absorption by plant and day were calculated (Urrestarazu et al., 2015). The nutritional solutions recommended by Sonneveld and Straver (1994) were used, to which 0.60 mM of Si (+Si) of the commercial product as orto potassium silicate FertiSil® (Macasa, Barcelona, Spain) was added; no Si product was added to the control (-Si).

### 2.2. Vegetative growth parameters

Vegetative development was measured 75 days after transplant and application of treatments. Biomass growth was measured through the dry and fresh weight of the roots, stems and leaves (g plant$^{-1}$) and leaf area (m$^2$ plant$^{-1}$).

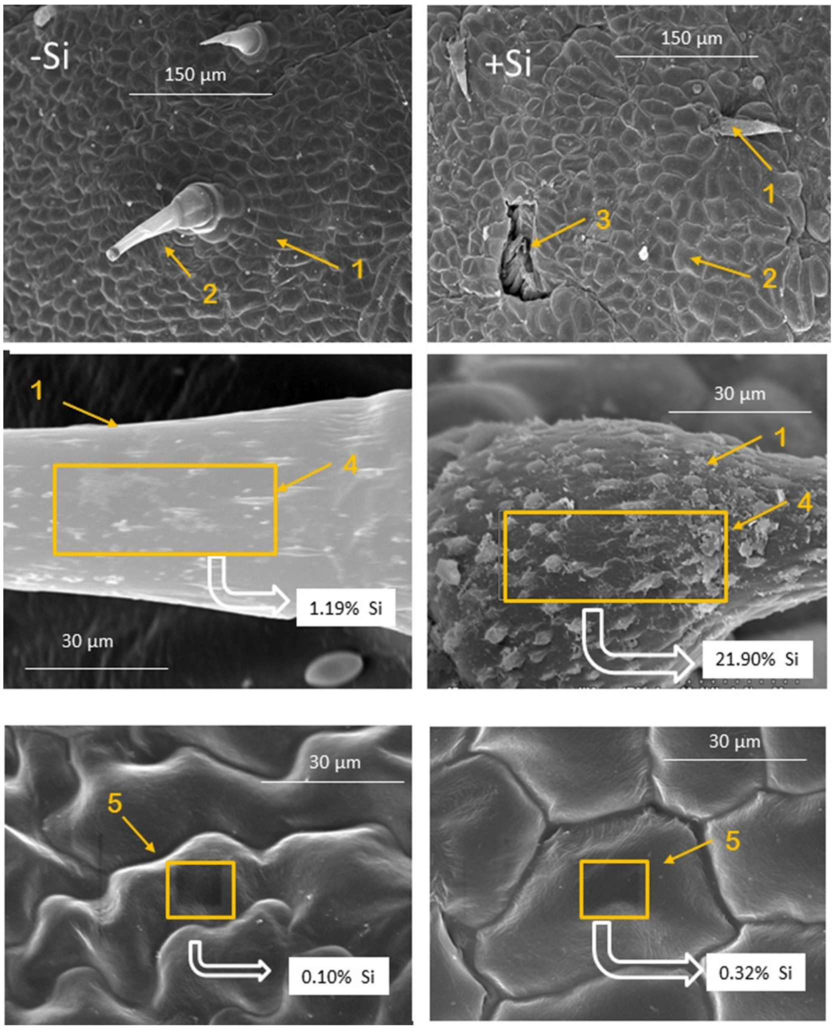

**Fig. 2.** Scanning electron microscopy images of pepper leaf treated with Si in the nutrient solution (+Si) (second column) versus a control plant (-Si) (first column). 1 and 2 are trichome and epidermal cells on surface leaves. 3 is epicuticular crystals on the adaxial surface of the leaf. 4 and 5 are yellow micro-areas of the trichome or epidermal cells where X-ray microanalysis was performed.

### *2.3. Test for resistance to deformation and breakage in leaves*

The leaf firmness was determined by penetrometry at the end of the experiment by a digital diameter penetrometer (53200-Samar, Tr-Turoni, Forli, Italy) with a 6 mm diameter drill. The results were expressed in Newtons. Resistance to deformation was analyzed by nondestructive measurements of the leaves with a hardness tester (Durofel, Copa Technology, France) equipped with a 0.1 $cm^2$ tip. A scale of 1 (soft) to 80 (firm) in Shore degrees is used. Four measurements were taken in the middle of each leaf for 10 leaves per replication.

### *2.4. Measurement of moisture loss in leaves*

For the measurement of the loss of moisture from the leaves at the end of the experiment, a sample of four leaves was taken per plant, repetition and treatment. They were sealed by their petiole by

**Table 1**
Water absorption (L $plant^{-1}day^{-1}$), nitrates and potassium (mmol $plant^{-1}$ $day^{-1}$) in horticultural crops for a period of 50 days of treatment after transplant. +Si indicates the application of Silicon in the standard nutrient solution (-Si) with an optimal fertigation (OF) and deficit (DF).

| | Cucumber | | | Melon | | | Pepper | | |
|---|---|---|---|---|---|---|---|---|---|
| | Water | $K^+$ | $NO_3^-$ | Water | $K^+$ | $NO_3^-$ | Water | $K^+$ | $NO_3^-$ |
| OF -Si | 0.411b | 0.209c | 0.210b | 0.415b | 0.514b | 0.367c | 0.386b | 0.200b | 0.212b |
| +Si | 0.455a | 0.283b | 0.395a | 0.449a | 0.770a | 0.737a | 0.431a | 0.275a | 0.382a |
| DF-Si | 0.340d | 0.160d | 0.177c | 0.347c | 0.398c | 0.334c | 0.317c | 0.196b | 0.150c |
| +Si | 0.381c | 0.309a | 0.219ab | 0.382b | 0.533b | 0.391b | 0.339bc | 0.280a | 0.391a |

Different letters indicate significant differences at $P \leq 0.05$ according to Tukey test.

immersion in paraffin and were weighed on a precision scale to the thousandth of a gram. Then, they were placed in a forced air stove (OMS60, Thermo Scientific®, USA) at 40 °C and weighed again after 10, 30, 60 and 360 min. Leaf water loss data were expressed in mL $cm^{-2}$ $hour^{-1}$.

### 2.5. *Test for protection against gray rot and powdery mildew*

The infecting biological materials were *Botrytis cinerea* (gray rot) and *Erysiphe cichoracearum* (powdery mildew).

### 2.6. *Assay to evaluate benefits against gray rot*

From each treatment, 4 representative leaves of each crop and treatment were taken. Four discs were extracted from each leaf and taken to a controlled environment for their corresponding inoculation (if applicable), measurement and evaluation of the growth of *B. cinerea* following the procedure described by Wegulo and Vilchez (2007) and (Pozo et al., 2015). The discs extracted from + Si and -Si plants were deposited in Petri dishes boxes. At 3, 7 and 14 days after inoculation, the progression of the disease was observed and recorded as a percentage of the affected surface. For the measurement of the affected surface, the WinDIAS® program (Delta-T Devices Ltd, UK) for interpretation and analysis of images was used.

### 2.7. *Assay to evaluate powdery mildew infection*

Four cucumber plants treated with + Si and the control were taken to a controlled environment with a humidity above 85 %, and a spraying of the causal agent of powdery mildew (*Erysiphe cichoracearum*) was performed. After 10 and 15 days, the central leaves of four plants per treatment were sampled. By taking photographs, the affected area (%) was quantified using the ImageJ® (Rueden et al., 2017), image processing program. Calculation of the apparent infection rate as a function of the application the logistic model by Berger (1980), was performed based on the equation:

$$y = 1/(1 + \exp(-[a + rt{+}rt]))$$

where y = the disease proportion in the range $0 < y < 1$, a = logit (*yo*), r = rate, and t = time.

### 2.8. *Optical and electron microscopy*

Four leaves were sampled from the middle part of the plants of each crop and treatment. Each leaf was fixed (Sargent, 1976), for later observation under optical and electron microscopes. Quantification of trichomes was performed using a light microscope (Nikon Eclipse E800, Japan) and a HITACHI S-3500 N LV-SEM (®Hitachi High-Technologies Corporation, Japan) for scanning electron microscopy (SEM) (Fig. 1). The chemical analysis of the sample inside the SEM was performed by Energy Dispersive Spectrosopy (EDS) with INCAx-sight (Oxford, England) using 180 and 320 $\mu m^2$ for epidermal cells and trichomes, respectively (Fig. 2).

### 2.9. *Experimental design and statistical analysis*

The experiment was carried out following a randomized complete block experimental design (Petersen, 1994). Statistical analysis was performed using ANOVA and Tukey's test with the program STATGRAPHICS PLUS version 5.0.

**Table 2**
Leaf area ($m^2$ $plant^{-1}$) and biomass (g $plant^{-1}$) 50 days after transplant in a horticultural crop. +Si indicates the application of Silicon in the standard nutrient solution (-Si) with optimal fertigation (OF) and deficit (DF).

| | Cucumber | | | | Melon | | | | Sweet pepper | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Leaf area | | | | | | | | | | | |
| OF -Si | | | | 1.71c | | | | 1.83b | | | | 1.78c |
| +Si | | | | 2.32a | | | | 2.85a | | | | 2.47b |
| DF -Si | | | | 1.63c | | | | 1.77c | | | | 1.72d |
| +Si | | | | 2.22b | | | | 2.70a | | | | 2.49b |
| | Root | Stem | Leaf | Total | Root | Stem | Leaf | Total | Root | Steam | Leaf | Total |
| | Fresh weight | | | | | | | | | | | |
| OF -Si | 16.16c | 52.70b | 53.84bc | 122.70c | 58.74b | 155.61b | 123.44c | 337.79c | 40.64c | 52.15c | 47.47b | 140.26b |
| +Si | 26.87a | 88.36a | 162.46a | 277.69a | 63.92a | 237.53a | 187.88a | 489.33a | 70.12a | 91.01a | 60.06a | 221.19a |
| DF -Si | 11.28d | 25.57c | 35.73c | 72.58d | 51.83b | 137.29c | 109.49d | 298.61d | 38.19c | 27.39d | 46.91b | 112.49c |
| +Si | 22.44b | 82.42a | 86.76b | 191.62b | 63.13a | 199.28ab | 166.15b | 428.86b | 68.24b | 81.30b | 67.94a | 217.48a |
| | Dry weight | | | | | | | | | | | |
| OF -Si | 0.82c | 17.10d | 12.04c | 29.96c | 10.08b | 28.27b | 25.37b | 67.98b | 11.56b | 15.10b | 14.67c | 43.77b |
| +Si | 1.19a | 22.78a | 36.20a | 60.17a | 15.14a | 35.66a | 37.10a | 82.70a | 20.12a | 27.48a | 16.98b | 49.81a |
| DF -Si | 0.70d | 8.56c | 11.00d | 20.26d | 7.59c | 22.15b | 22.37c | 53.11c | 12.83c | 9.47c | 14.00c | 33.13c |
| +Si | 0.93b | 19.39b | 21.75b | 40.07b | 13.91b | 37.80a | 31.60b | 80.22a | 19.81b | 23.67a | 18.60a | 45.08b |

Different letters indicate significant differences at $P \leq 0.05$ according to Tukey test.

**Table 3**
Thickness (μm) of adaxial (Ad) and abaxial (Ab) epidermis, adaxial cuticle (AdC), leaf lamina (LL) and trichomes (N° mm$^{-2}$) from scanning electron microscope section after 75 days of Si (+Si) versus a control plant (-Si).

| | Cucumber | | | | | Melon | | | | | Pepper | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Trichome | Ad | Ab | AdC | LL | Trichome | Ad | Ab | AdC | LL | Trichome | Ad | Ab | AdC | LL |
| -Si | 33b | 17.90b | 28.96a | 0.60b | 137.48b | 30b | 20.83a | 29.89b | 0.56b | 134.30b | 16b | 17.25b | 27.51a | 0.58b | 128.65b |
| +Si | 39a | 21.42a | 29.69a | 0.64a | 177.36a | 36a | 21.56a | 33.39a | 0.70a | 173.48a | 20a | 18.68a | 27.79a | 0.61a | 164.83a |

Different letters indicate significant differences at $P \leq 0.05$ according to Tukey test. n = 4.

## 3. Results and discussion

### 3.1. Effect on fertigation parameters

With the application of Si in the nutrient solution (+Si) for the three crops tested and under optimal irrigation (OF) and deficit irrigation (DF), the absorption of water, nitrates and potassium improved by 10, 50 and 70 %, respectively (Table 1). Ma et al. (2001) reported that the presence of Si in the nutrient solution increased water absorption, protecting plants against abiotic stresses (Hernandez-Apaolaza, 2014). This improved absorption has also been described under saline conditions by Shi et al. (2016), when applying Si in the nutrient solution up to 2.5 mM.

With the addition of Si to DF, the absorption of potassium and nitrate in all crops was equal to or better than that in the OF -Si treatments. This suggests that the loss of ionic absorption under moderate water stress is alleviated by the incorporation of Si into the nutritive solution. These results agree with those of Kaya et al. (2006), who reported that silicon addition increased K levels in water-stressed maize leaves. It was reported that increased uptake of K may be attributed to a decrease in plasma membrane permeability and an increase in plasma membrane H+-ATP activity as a result of silicon addition (Liang, 1999).

Likewise, +Si alleviated the decrease in water availability in the DF treatment, recording the same absorption of water as that in OF under -Si. Therefore, the contribution of Si also alleviated the lower water availability of the DF treatment. This result suggests that the improvement in the water balance of the plant is produced not only by the decrease in transpiration due to the potential greater thickness of the cuticle but also by the improvement in water absorption by the roots of cucumber, melon and pepper. In tomato plants, Shi et al. (2016), reported that leaf water content under water stress was preserved when plants were supplied with Si, suggesting that increased water uptake/-transport rather than decreased transpiration was responsible for Si alleviating water stress.

### 3.2. Effect on vegetative growth parameters

In both OF and DF treatments, the addition of silicon in fertigation registered a clear and significant benefit in all growth parameters in the three crops, with an average increase of more than 20 % (Table 2). The benefit of silicon to the leaf area was similar under OF and DF, but the average Si benefit (more than 10 %) was higher under DF than under OF, suggesting that under stressful situations, the benefit of applying Si in the nutrient solution is greater than that under optimal fertigation conditions. Cucumber cultivation showed an average significant increase twice as high as that found in melon and pepper. This stronger beneficial effect of Si on cucumber can justify why authors such as Sonneveld and Straver (1994), recommend its use in cucurbits such as cucumber and melon and do recommend Si for pepper. However, Pozo et al. (2015), reported similar results to ours in lettuce, tomato, pepper, melon and cucumber plants grown in coconut fiber.

In the -Si treatments, the foliar area of melon and pepper in DF was significantly lower than that of melon and pepper grown with Si in the nutrient solution. The + Si treatments always significantly increased the leaf area with respect to that in the controls.

For DF and -Si, the total fresh and dry weight of the three crops tested was also significantly lower, by an average of 20 %. In DF treatments in which Si was applied, this reduction would disappear in pepper and decrease to an average of 14 % for cucumber and melon.

For all vegetative growth parameters evaluated, the DF and + Si treatments obtained results that were the same as or better than those in the OF and -Si treatments. Therefore, the presence of Si in the nutrient solution alleviated hydric stress. Our results suggest an important mitigation of losses from moderate water stress, as has been reported in wheat (Meunier et al., 2017), cucumber (Adaita and Besford, 1986; Hattori et al., 2008), and pepper (Lobato et al., 2009).

### 3.3. Effect on trichomes

The number of trichomes in cucumber and melon was similar (≈ 30–40 per mm$^{-2}$) and was lower in pepper (Table 3). This density is similar to the reported number of glandular trichomes in the trichome plants *Ocimum campechianum* and *Ruellia nudiflora* (Martínez-Natarén et al., 2018). The number of trichomes in the three species was increased by 25 % when Si was used in the nutrient solution. An increase in trichome number in soybean plants has been reported when applying 0.8 mM of Si (Tibbitts, 2016), and a significant improvement in trichome number was found when applying 1.5 mM of Si (Liang, 1999). Incorporating Si up to 1 mM did not increase the number of trichomes on cucumber fruits, but the trichomes under + Si had a coarse outer appearance compared to those under -Si, where the trichomes were smooth (Samuels et al., 1993).

### 3.4. Effect on foliar anatomy

The total thickness of the leaf was similar among the three plants tested. The + Si treatment thickened leaves very significantly, by 30 %, an increase that had already been shown in the highest dry and fresh leaf weight. In the case of cucumber and pepper, the thickness of the epidermal layer of the leaf bundle increased significantly, by 20 and 9%, respectively, while in melon, the abaxial epidermis increased by 10 %, compared to those in the -Si treatments.

### 3.5. Effect on the cuticle

Cuticle thickness was very similar in all three plants and was within the mean values described by Tafolla-Arellano et al. (2018) and Tafolla-Arellano et al. (2013). With the addition of Si to the nutrient solution, the cuticle recorded an increase of 20, 9 and 5% in cucumber, pepper and melon, respectively. With the same concentration of Si in the nutrient solution, Pozo et al. (2015) found similar increases in tomato, cucumber, pepper, melon and lettuce plants. Therefore, the benefits of growth may be partly due to this thickening of the cuticle (Van Bockhaven et al., 2013).

### 3.6. Effect on the microanalytic concentration of Si in the cuticle

Hernandez-Apaolaza (2014) have been reported different Si translocation rates to the leaves by the xylem in higher plants. However, specific via to deposit Si on the leaves need further investigation (Savvas and Ntatsi, 2015). Fig. 3 shows the Si content in the cuticle and trichomes of the tested horticultural plants. With the exception of the melon trichomes, the + Si recorded significant increases in Si content in

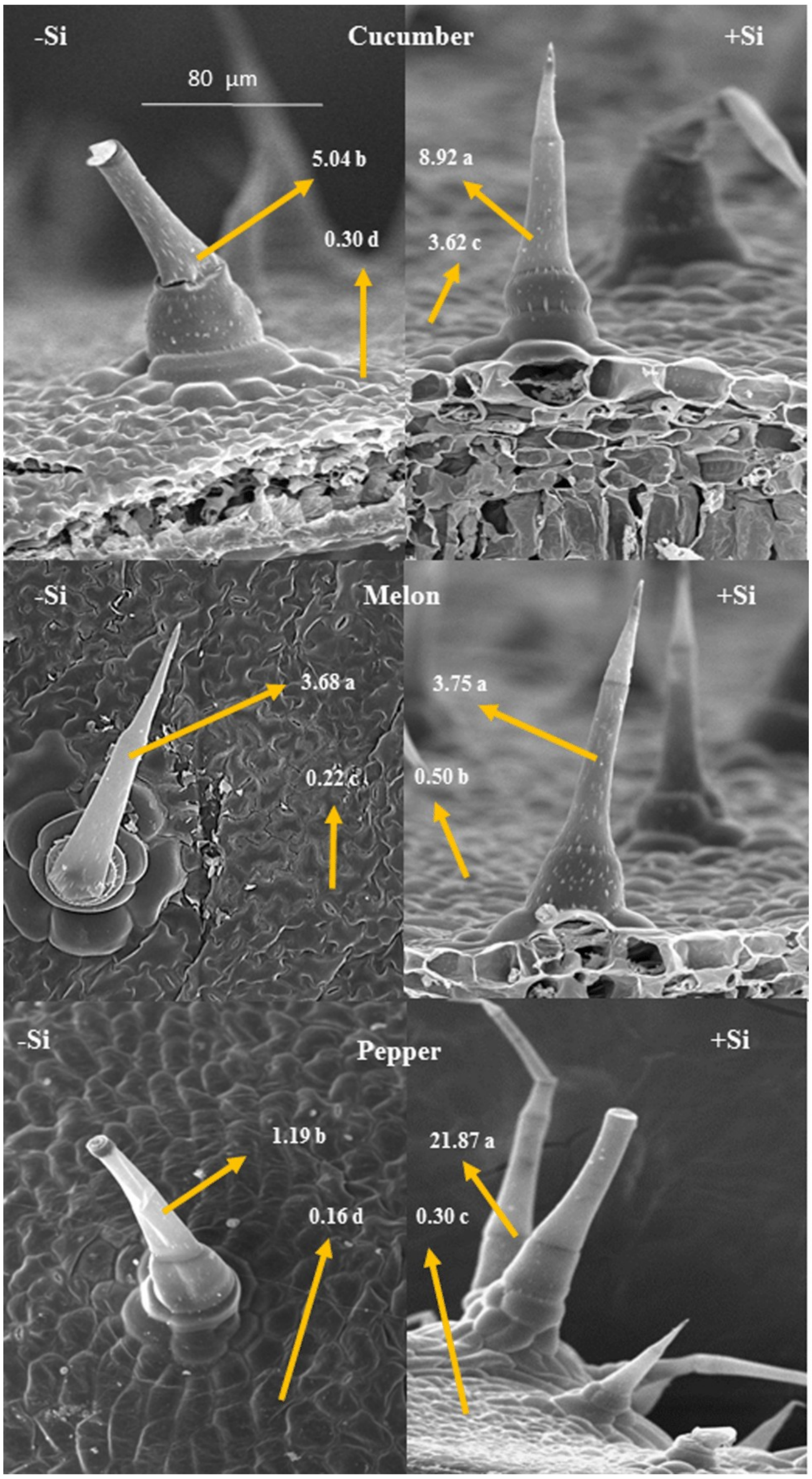

**Fig. 3.** Si concentration (%) in the different cultivars measured by scanning electron microscopy. Different letters indicate significant differences at $P \leq 0.05$ according to Tukey's test. n = 4.

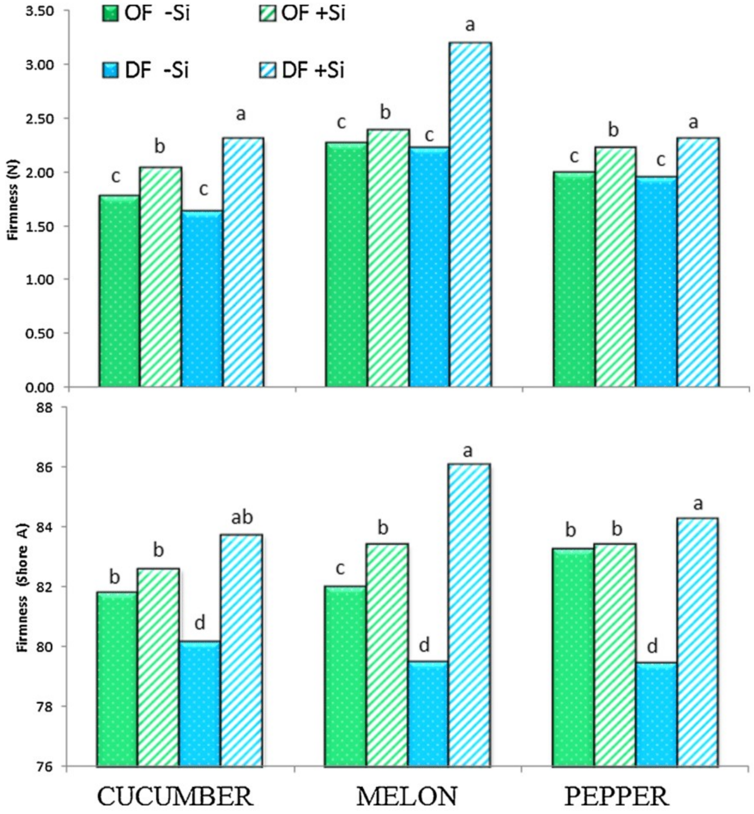

**Fig. 4.** Resistance to deformation (Shore) and firmness (N) of the leaves with the application of Si in the nutrient solution in pepper, melon and cucumber crops. +Si indicates the application of silicon in the standard nutrient solution (-Si) under optimal (OF) and deficit (DF) fertigation. Different letters indicate significant differences at $P \leq 0.05$ according to Tukey's test. n = 4.

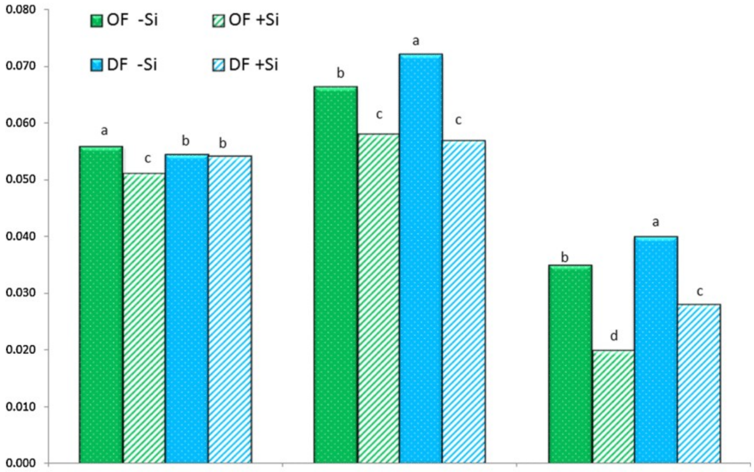

**Fig. 5.** The leaf desiccation rate (ml $cm^{-2}$ $hour^{-1}$) with the application of Si in the nutrient solution in pepper, melon and cucumber crops. +Si indicates the application of silicon in the standard nutrient solution (-Si) under optimal (OF) and deficit (DF) fertigation. Different letters indicate significant differences at $P \leq 0.05$ according to Tukey's test. n = 4.

both trichomes and the cuticle compared to those in the -Si treatments, and phytoliths and oxalate crystals were only observed in the + Si treatments. In the case of cucumber and pepper, the trichomes had a significantly higher Si concentration (>75 %) than those under the -Si treatments. Many studies have reported a higher presence of Si in the external layers of the leaves when Si is applied in the nutrient solution

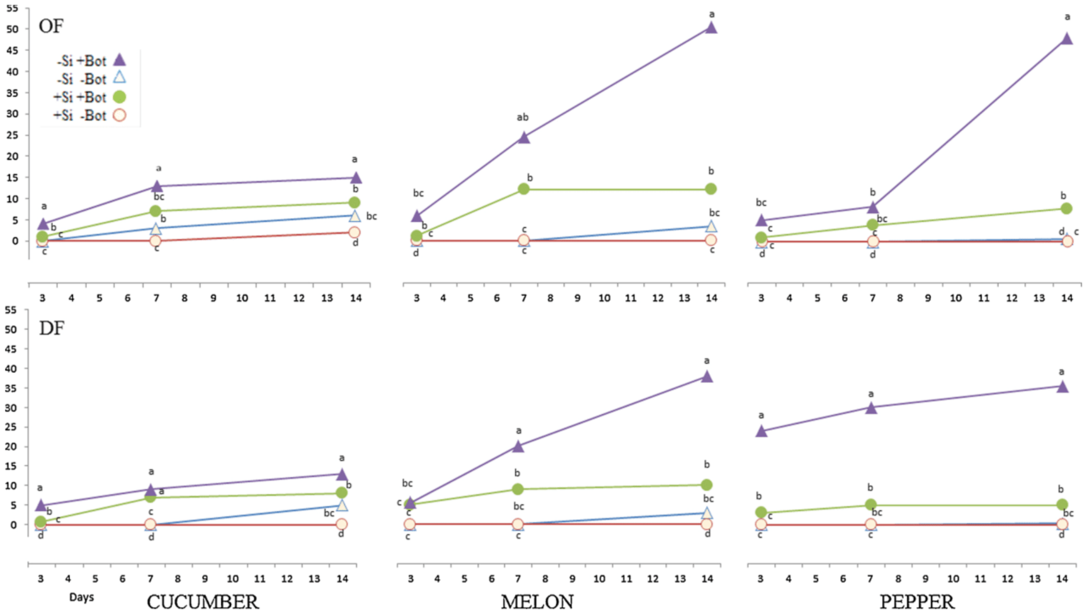

**Fig. 6.** Severity of gray rot disease over time on leaf discs expressed as the percentage of the infected area. +Si + Bot, −Si + Bot, +Si − Bot, and − Si − Bot refer to discs from plant growth in the nutrient solutions with silicon (Si) and with *Botrytis cinerea* inoculum, without Si and with *B. cinerea* inoculum, with Si and without *B. cinerea* inoculum, and without Si and without *B. cinerea* inoculum, respectively. Different letters indicate significant differences T P ≤ 0.05 at same time after inoculation according to Tukey's test. OF and DF indicate optimun and deficit fertigation, respectively.

(Ma and Takahashi, 2002). Although the concentration of Si was much lower when Si was not applied in the nutrient solution, a higher proportion of Si was found in all cases in the trichome than in the cuticles, suggesting that Si is preferably mobilized to the trichomes themselves for formation. Meunier et al. (2017), described the formation of the highest number of trichomes with the presence of Si in the nutrient solution. However, the metabolic mechanism of Si translocation to the trichomes requires further study.

### 3.7. Effect on leaf firmness

Fig. 4 shows the effect on the mechanical resistance of the leaves depending on the type of fertigation and the application of Si. It is well known that Si contributes to a higher mechanical resistance in the leaves (Tafolla-Arellano et al., 2018, 2013; Kaufman et al., 1979; Sahebi et al., 2015). Our results show that the highest mechanical resistance, deformation resistance and resistance to leaf rupture always occurred with Si application and under DF. With the exception of the OF treatment in pepper, the presence of Si reduced deformation by 15 % and reduced penetration by 25 %. This greater resistance to deformation led to less deformation from wind (Debona et al., 2017; Tafolla-Arellano et al., 2018; Martínez-Natarén et al., 2018), and therefore can partly explain the better vegetative growth, probably because the leaves remained intact during a longer period of exposure to light.

### 3.8. Effect on leaf drying

With the exception of the DF treatment in cucumber, the application of Si led to a lower loss of moisture through the cuticle (Fig. 5). The reduction in water loss was directly proportional to the cuticle thickness of the three cv. tested. This decrease in water loss from the leaves was also recorded by Tibbitts (2016). This result also agrees with the well-known decrease in transpiration that occurs when Si is supplied (Ma, 1990; Tafolla-Arellano et al., 2018).

**Table 4**
Apparent rate of infection and % affectation of the leaves by Oidio (*Erysiphe cichoracearum)* on a cucumber crop. +Si indicates the application of Silicon in the standard nutrient solution (-Si) with an optimal fertigation (OF) and deficit (DF).

| | | % Rate of infection | Nº plants affected within 15 days | Apparent rate of infection |
|---|---|---|---|---|
| OF | -Si | 7.37a | 5.3b | 0.0294a |
| | +Si | 2.13b | 5.5b | 0.0119bc |
| DF | -Si | 8.79a | 7.1a | 0.0156b |
| | +Si | 3.71b | 6.4b | 0.0088c |

Different letters indicate significant differences at P ≤ 0.05 according to Tukey test. n = 4.

The results suggest that the addition of Si to the nutrient solution produces 1) a decrease in water loss from the leaves and therefore lower desiccation, justified in part by an increase in the thickness of the cuticle and epidermis; and 2) a greater absorption of water, which improves the hydric balance and explains in part the improvement in the vegetative development. These results are in agreement with the results reported in tomato by Romero-Aranda et al. (2006) and Haghighi and Pessarakli (2013), and in potato by Shi et al. (2016).

### 3.9. Effect of Si on plant protection

It is well known that the leaves Si depositions protect plants of multiple abiotic and biotic stresses (Datnoff et al., 2001; Ma and Takahashi, 2002; Ma and Yamaji, 2006; Tafolla-Arellano et al., 2018). Fig. 6 shows the protective effect of the addition of Si to the nutrient solution against the pathogen *Botrytis cinerea*. From 7 days after inoculation, significant differences appeared in the progression of gray rot in the inoculated discs of the three horticultural plants tested compared to that in the noninoculated plants. The trends in OF and DF were analogous. These results were very similar to those found by Pozo et al. (2015), in

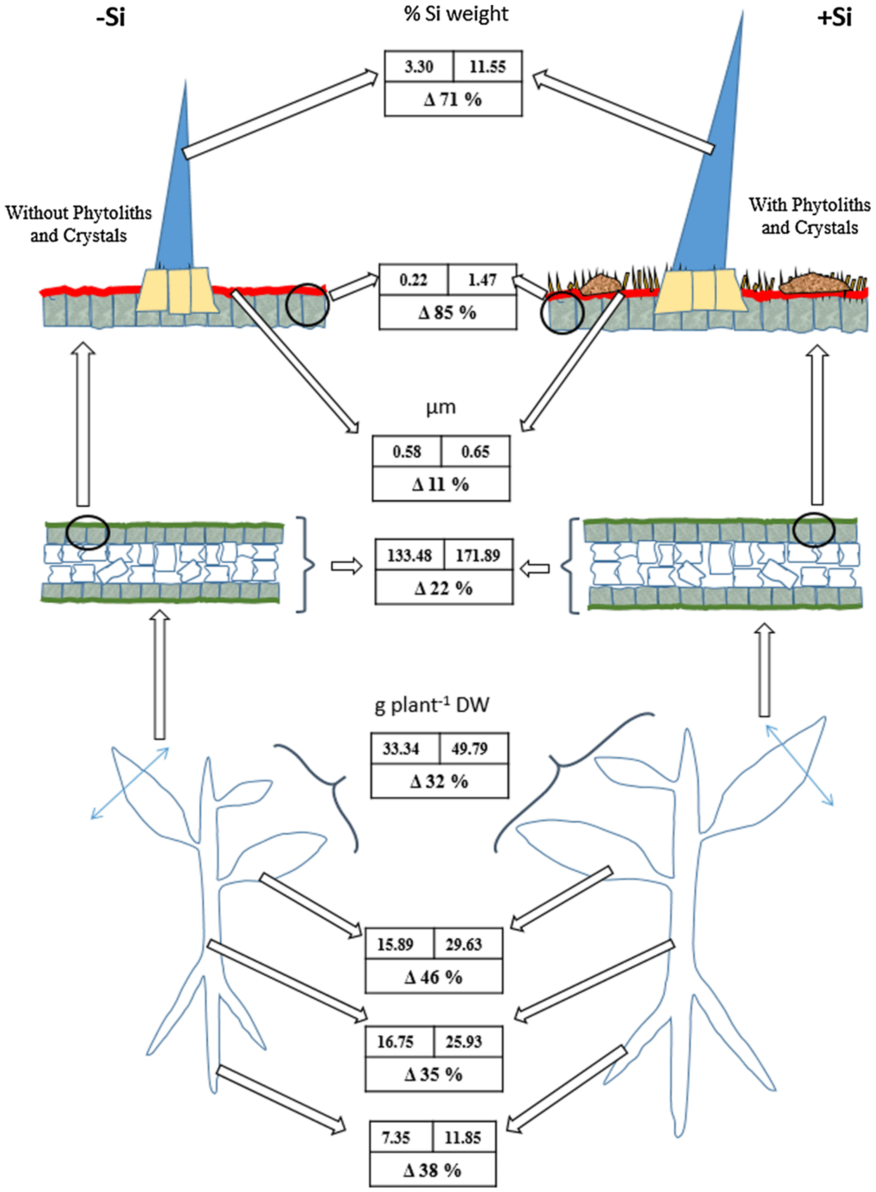

**Fig. 7.** Averages of the effects on growth and Si concentration in the cuticle and trichomes in vegetable plants treated with Si in nutrient solution (+Si) versus the effects of a control (-Si).

the same horticultural plants and with the same dose of Si in the nutrient solution. The high cuticle thickness described above in the tested horticultural plants exerted greater protection against the pathogenic agent *B. cinera.*

In both OF and DF, the presence of Si in the nutrient solution in the cucumber crop exerted a significant protective effect against affectation in the leaves and in the apparent affectation rate (Table 4). As with the protection against the pathogenic agent *B. cinerea*, the increased cuticle thickness of the cucumber crop under + Si produced a protective effect against powdery mildew. This effect of silicon in soilless culture had also been reported by Adaita and Besford (1986), in cucumber protection against powdery mildew.

In general, our results suggest better benefit under stress condition, this is in accordance with reported by Hernandez-Apaolaza (2014): "its beneficial effects are usually expressed more clearly when plants are subjected to various abiotic and biotic stresses".

## 4. Conclusion

The addition of Si to the nutrient solution improves plant growth. We suggest that, in addition to the metabolic implications, this improvement may be for two reasons: 1) The water balance of the cucumber, melon and pepper plants is improved by the Si in the nutrient solution both a) by the improvements in water absorption by the roots and in transport towards the leaves, and b) by the lower water loss through the cuticle due to its improved thickness; and 2) The improved turgidity (point 1) and the rigidity conferred by the thicker cuticle and the phytoliths and trichomes allow the leaf to spend a longer time being exposed to light. The application of Si alleviated the loss of growth caused by moderate deficit irrigation. The Si transported by the xylem clearly preferentially accumulates in the trichomes, which implies active transport towards these structures; the number of trichomes also increases when Si is applied through fertigation (Fig. 7). The thicker cuticle and consequently the more rigid leaves increase plant resistance to diseases that need to cross an epidermal barrier.

## CRediT authorship contribution statement

**Francisca Ferrón-Carrillo:** Methodology, Formal analysis, Resources. **Miguel Urrestarazu:** Conceptualization, Methodology, Writing - original draft, Writing - review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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# 2. Silicon enhances production and quality of blueberry fruits

# Silicon enhances production and quality of blueberry fruits

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## Abstract

*The effect of silicon (Si) in the nutrient solution on the fruit development of 2-year-old blueberry plants (Vaccinium corymbosum L. cv. Ventura) was studied. Si was applied to the nutrient solution at a dose of 0.0, 0.6, and 1.2 mM. The parameters of fruit, stems and leaves growth, firmness, and biomass were measured. Fertigation in conjunction with traditional spread fertilization could improve the growth and yield of highbush blueberry to find the optimal method to control decay and prolong the quality of blueberries after harvest. The blueberry fruit has a light-blue appearance because its blue-black skin is covered with a waxy bloom. This layer is easily damaged or removed during fruit harvesting and postharvest handling. Si enhances the resistance of this layer to damage. A 2-year study was done to compare the effects of silicon fertigation and silicean sand and coir fibre as substrates on growth and availability of nutrients for blueberry plants during establishment of highbush blueberry (Vaccinium corymbosum L. "Bluecrop"). The results of experiment indicated that the application of Si had better benefit on the fruit growth of blueberry plants in coir fibre, than the effect that was observed in the sand substrate. The results should improve our understanding for better preservation of postharvest quality of blueberry fruit.*

**Keywords:** blueberry fruits, firmness, sand, coir fibre, silicon.

## Introduction

Because of their unique taste, delicate, high nutritional value, and beneficial effects on human health, fresh blueberries (*Vaccinium* spp.) have become one of the most widely consumed fruit and a new popular functional food worldwide (Bornsek et al., 2012; Kong et al., 2014). In addition, its cultivation has become interesting, due to the high profitability (Strik and Yarborough, 2005; Bañados, 2006; Caspersen et al., 2016).

According to the Food and Agriculture Organization of the United Nations (FAO, 2018), in recent years, there has been an increase in the blueberry cultivation area of around 34%, corresponding to 28,000 ha and a production of 552,505 t, with the United States and Canada being the main producers. Spain, with 6412 t, is the eighth largest producer and has become one of the most important producers worldwide.

Fresh blueberries are also highly perishable, with a short shelf life as a result of field heat, mechanical damage, postharvest respiration, microbial decay, and moisture and nutritional loss (Zhou et al., 2014). The fruit quickly soften after storage at 0 °C, due to the damage of cuticular wax and sweating (Gao et al., 2015). However, one of the main factors influencing the development and production of crops is the pH of the soil or the substrate, and the nutrient solution, which can interfere with the availability of nutrients

(Urrestarazu, 2015). It is well known that blueberries develop well in acidic soils with a pH of 4.5 to 5.5 and with low levels of fertility (Retamales and Hancock, 2012).

Even though it is not an essential element, the benefits of silicon (Si) have been studied both in plant protection (Urrestarazu et al., 2016) and in mineral nutrition (Zhu and Gong, 2014). In addition, other proven beneficial effects of Si are the reduction of water loss in the cuticle due to accumulation in the epidermis (Pozo et al., 2015; Gallegos-Cedillo et al., 2018).

The outermost layer of plants is covered with cuticular wax, which is visible as a white or bluish coating on the surface of some fruits, such as blueberry, plum, and grape (Wisuthiphaet et al., 2014). Cuticular wax is the first protective barrier against biotic and abiotic stresses; it plays a vital role in limiting the non-stomatal water loss and in preventing the spore germination of pathogenic microbes (Bernard and Joubès, 2013). In recent years, several studies have demonstrated that cuticular wax is closely related to the postharvest quality of fruit (Martin and Rose, 2014).

Softening is also an important factor of quality deterioration after in blueberry fruit harvest (Angelerrib et al., 2010). Fruit softening is due to cell wall degradation by cell wall-associated hydrolysis enzyme activities (Wang et al., 2017). The Si has been published as a plant-beneficial element associated with the mitigation of abiotic and biotic stresses (Boldt et al., 2018). However, there is no information about the proper levels of Si in fertigation and neither about the possible benefits in the cultivation of the blueberry (Bryla and Strik, 2015; Vargas et al., 2015; Urrestarazu et al., 2018).

The general objective of this work was to evaluate the effect of the nutrient solution applying different concentrations of silicon, on the production and quality of blueberry fruit in a soilless culture system with silica sand and coconut fiber substrate. The secondary objective was to measure the level of silicon present in it and it effect on the organoleptic quality of the fruit in soilless culture production and enhance its postharvest life.

## Material and Methods

### ***Treatments and growth conditions***

Experiment was carried out at the University of Almería, Spain, in a multispan greenhouse with characteristics that have been described by Valera et al. (2016). On 1 Apr. 2017, 2-year-old blueberry plants (*Vaccinium corymbosum* L. cv. Ventura) were transplanted from 1-L containers to 22-L containers with Pelemix CF substrate, the physical characteristics of which have been described by Rodríguez et al. (2014), and silica sand with a granulometry of 0.5 to 1.0 mm in diameter. Vegetative development was evaluated on 9 March. 2018. The planting density was 2.5 plants m$^{-2}$.

### ***Application of silicon to the nutrient solution.***

The plants were fertigated with a nutrient solution similar to that proposed by Sonneveld and Straver (1994). The Si treatments were 0.0, 0.6, and 1.2 mM. The Si source used was Fertisil (MACASA, Barcelona, Spain). The pH of the nutrient solutions was adjusted to 5.5 with dilute nitric acid.

The irrigation for this crop is done with deionized water. This type of water has been removed by ion exchange resins of mixed bed, cations such as sodium (Na), calcium (Ca), iron (Fe), copper (Cu), and anions such as carbonate, fluoride, chloride, among others. While blueberry requires for good development and production, irrigation water pH < 7.0 and bicarbonate ($HCO_3^-$) < 1.5 mmol $L^{-1}$, sodium ($Na^+$) < 2.0 mmol $L^{-1}$ and chloride (Cl) < 4.0 mmol $L^{-1}$ (Ferreyra et al., 2001).

***Fertigation scheduling.***

Each new fertigation was performed when 10% of the readily available water was used up (Urrestarazu, 2015; Urrestarazu et al., 2017). The EC of the nutrient solutions always remained between 1.5 and 1.6 dS $m^{-1}$. Fertigation management and growth parameters were performed. The pH and EC of the supplied nutrient solution and the drainages were monitored daily using a Crison MM40+ Conductivimeter and pH meter (LPV2500.98.0002; Hach, Vizcaya, Spain), and the volume (measured in liters) was determined with a graduated cylinder with 100 mL of precision. Based on the irrigation and drainage volumes, the water uptake was estimated according to Pozo et al. (2014).

***Parameters of vegetative growth.***

Training pruning was performed 203 d after the application of the treatments, which were used to evaluate vegetative development. The parameters of vegetative and fruit growth measured were plant height (measured in meters), polar and equatorial fruits diameter (mm), shoot biomass (leaves, stems and fruits), ºBrix of fruits (measured by a PS PRO refractometer), and leaf area (measured in square meters per plant), as measured with an AM350 Area Meter (ADC BioScientific Ltd., Hertfordshire, UK). The plants were divided by their different organs, and stem and leaf fresh weights were obtained. The dry weight was obtained by placing the material in a convection oven (Thermo Scientific Heratherm, Germany) at 75 ºC until constant weight and then measured using an OHAUS Adventurer Analytical Precision Analytical Balance (model AX 124/E). The pH was adjusted with nitric acid.

***Parameters of firmness and resistance to breackage.***

The resistance of leaves and fruits to deformation was measured in samples of 10-15 leaves per replica, by deformation of these using a hardness tester (Durofel, Copa Technology, France) equipped with a 0.1 $cm^2$ tip on a scale from 1 (soft) to 60 (signature). Durometer readings were equivalent to the force in Newtons as determined by a linear model (Ynewton = 0.47 + 0.077 Durofel reading; R2 = 0.97). The data are presented in Newtons and the relationship was similar to that described by Polenta et al. (2006).

The resistance to breakage of leaves and fruits (firmness) was determined using a manual digital penetrometer (Samar 53200; TR-Turoni, Italy) fixed in a support drill with a tip of 6 mm in diameter. The penetration depth was 9 mm in the pulp, and the results are expressed in Newtons (N). The readings were taken from the sides of the leaf with one reading per leaf using four replicas of 10 plants taken randomly from each treatment group.

***Statistical analysis and experimental design.***

The experiment was performed using a randomized complete block design, with four repetitions and four plants per repetition for each treatment in CF and sand (Montgomery, 2004). The results were subjected to an analysis of variance and a comparison of means with a Tukey test ($P \leq 0.05$), and to linear and quadratic correlations using the statistical package Statgraphics Centurion X.V.I.I (2018).

## Results and Discussion

### *Effect on fertigation parameters*

With the application of Si in the nutrient solution with a concentration of 0.0, 0.6 and 1.2 mM respectively, for the crop tested in coir fiber (CF) and silica sand (SIS), the absorption of water, nitrates and potassium improved as shown in Table 1. For coir fibre, treatment with addition of Si to the nutrient solution at a dose of 1.2 mM registered the best values for $K^+$, $NO_3^-$ and water with (0.306, 0.364 mmol plant$^{-1}$ day$^{-1}$ and 0.431 L plant$^{-1}$day$^{-1}$ respectively). These values were better than showed by treatment using silica sand as substrate for blueberry production in this experiment, this tendency matches with research done by Urrestarazu et al. (2018) in blueberry cv. Ventura. This fact may be due to blueberry crop, despite being an acidophilus crop, is well influenced only in just some doses of Si, but when exceed, the effect should cause a negative impact on fruit quality.

Values of nitrates were significantly better in fruits subjected to treatments with addition of Si to the nutrient solution. These results are shown due to despite nitrogen deficiencies are the most common nutrient deficiency in blueberries worldwide (Hanson and Hancock, 1996; Hart et al., 2006), the negative effects of an excess in nitrogen fertilization, can be mitigated by the rigidity that confers the contribution of silicon (Guntzer et al., 2012).

This meant a significant improvement respect to treatment without addition of Si to the nutrient solution, which showed values for $K^+$, $NO_3^-$ and water of (0.245, 0.302 mmol plant$^{-1}$ day$^{-1}$ and 0.322 L plant$^{-1}$day$^{-1}$respectively).

Addition of Si at a dose of 0.6 mM to the nutrient solution did not reached higher values than treatment with addition of 1.2 mM to the nutrient solution. Urrestarazu et al. (2018) had already reported that the presence of Si in the nutrient solution and the use of coir fiber as substrate benefited water absorption with similar values (0.400 L plant$^{-1}$day$^{-1}$ respectively) by adding 0.6 mM Si to nutrient solution for the same species of plants.

Regarding silica sand as substrate, values followed the order 0 mM < 0.6 mM < 1.2 mM for showing the most significant values. This was specially the case for water absorption of plants influenced by treatment 1.2 mM, reaching values of 0.447 L plant$^{-1}$ day$^{-1}$ respect to treatment with addition of 0.6 mM to the nutrient solution with 0.384 L plant$^{-1}$day$^{-1}$. Moreover, potassium and nitrates absorption showed a correlation between water absorption values, following the tendency of register a better growth when Si was applied.

Potassium values were lower in treatments using silicean sand as substrate, research done by (Hart et al., 2006) matches with this tendency, explaining levels are rarely low in blueberries, except in sandy soils (Hart et al., 2006). Treatment without addition of Si had 0.200 mmol plant$^{-1}$ day$^{-1}$ respect to treatment with addition of 1.2 mM of Si (0.292 mmol plant$^{-1}$ day$^{-1}$).

This improved absorption had also been described in saline conditions by Ferrón-Carrillo et al. (2019) when applying Si in the nutrient solution up to 0.6 mM registering values of 0.283 and 0.395 mmol plant$^{-1}$ day$^{-1}$ for potassium and nitrates absorption . With the contribution of Si in CF the absorption of potassium and nitrate was greater than the treatments SIS. These results also explain the mitigation of stress thanks to the application of Si on the nutrient solution, as studied by (Boldt et al., 2018).

### ***Effect on vegetative growth parameters***

Regarding coir fibre, the number of leaves increased when applying Si to nutrient solution. Treatment with addition of 1.2 mM for coir fibre registered 420 leaves in total compared to 0.6 Mm Si with 248 leaves. These values, might be registered because of Si enhances the cuticle of the steam and leaves, enhancing their production.

In both CF and SIS, the addition of silicon in fertigation registered a clear and significant benefit on all growth parameters in the three concentrations 0.0, 0.6 and 1.2 mM (Table 2). This proves the benefits of the addition of Si in the nutrient solution for the growth of vegetative parameters, as results obtained by Ferrón-Carrillo et al. (2019).

For all vegetative growth parameters evaluated, the CF and 1.2 mM treatment obtained the same or even better results than the SIS and 1.2 mM treatment. Therefore, the presence of Si in the nutrient solution, these results match with data obtained by Urrestarazu et al. (2018).

As regard of silicean sand, the same line was followed by treatment with addition of 1.2 mM to nutrient solution, especially in total leaf area showing data of 11.90 $m^2$ $plant^{-1}$ respect to treatment without addition of Si with 7.40 $m^2$. Subsequently, Gomes et al. (2019), showed the same tendency in the yield of sauvignon blanc grape and wine, by addition of 8 g $L^{-1}$ of Si, with the enhancement of crop yield.

### ***Effect on leaf firmness***

When coir fibre was applied, Table 3 shows the effect on the mechanical resistance of the leaves depending on the type of fertigation and the different concentrations of application of Si, 0.0, 0.6 and 1.2 mM respectively.

It is well known that Si contributes to a higher mechanical resistance in the leaves, our results show that the highest mechanical resistance, deformation and rupture of the leaves, was always when applying 1.2 mM of Si and in the case of CF. With the exception of the 1.2 mM in SIS, which almost reaches the results of CF.

Besides, when silicean sand was applied, firmness and breakage were significant higher for treatment 1.2 mM in nutrient solution (90.4 Shore for 1.2 mM respect to 78.1 Shore for 0 mM in nutrient solution). These numbers may be the consequence of the silicon crystals inside the cuticle, making the leaves stronger.

Furthermore, breakage resistance showed values of 1.8, 2.5 and 3.6 N for treatments 0, 0.6 and 1.2 mM of Si. Moreover, Islam et al. (2018), showed an increase in fruit firmness in cherry tomato fruits, with 18.8 N of Control in comparison to treatment with addition of Si.

### *Effect on fruit growth parameters*

Blueberry cultivation in coir fiber showed an average significant increase twice as high as that found in SIC. This better behavior of the beneficial effect of Si cultivation can justify why authors such as Sonneveld and Straver (1994), recommend its use in crop.

Therefore, in coconut fibre Pozo et al. (2015) reported similar results to ours in lettuce, tomato, pepper, melon and cucumber plants. The treatments with 0 mM of Si on nutrient solution always significantly decreased the leaf area with respect to the treatments with Si, as reported by investigation obtained by Ferrón-Carrillo et al. (2019). This might be the consequence of the lack of phytoliths inside the cuticle of the fruits.

On the bright side, when silica sand used as substrate, the diameter of fruits experimented a growth, showing values of 13.1, 14.8 and 15.4 for treatments 0, 0.6 and 1.2 mM of Si. Fresh weight and dry weight also registered the same tendency, with the best values for 1.2 mM with 1.92 and 0.26 for fresh weight and dry weight (Table 4).

### *Effect on º Brix*

Figure 1 shows the effect of Si on the content of total soluble sugars (ºBrix) on blueberry fruits influenced by addition of Si to the nutrient solution.

Values of 10.33, 11.04 and 11.82 º Brix were recorded by treatments 0, 0.6 and 1.2 mM in coir fibre. Gomes et al. (2019), showed values of 19.1 ºBrix for treatments with addition of Si to nutrient solution respect to treatment without addition of Si with 20.1 ºBrix to nutrient solution with a value of 4 and 8 g $L^{-1}$ of Si.

This presented a correlation respect to treatments used in silican sand, with values of 10.38, 10.97 and 11.41 º Brix. Research done by Lozano et al. (2018), demonstrates the same tendency in melon fruits with values of 9.97 º Brix for a dose of 200 kg $ha^{-1}$ of Si.

Contrary to this data, Islam et al. (2018), recorded a decrease from 7.63 to 7.50 ºBrix on tomato fruits by addition of Si respect to control, this may be due to the fact they applied Si by foliar application, instead of directly to nutrient solution.

## Conclusions

The application of Si benefits the fruit and vegetative growth of blueberry plants which was better in coir fibre than in silica sand.

Fruits from plants influenced by Si at a dose of 1.2 mM in coir fibre registered higher resistance to craking and a better firmness.

Fruit weight was significantly increased by treatments with addition of 0.6 and 1.2 mM Si to nutrient solution.

Soluble solids º Brix in blueberry fruits enhanced their content by addition of Si at a dose of 1.2 mM to nutrient solution.

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**Table 1.** Water (L plant$^{-1}$ day$^{-1}$), nitrate and potassium (mmol plant$^{-1}$ day$^{-1}$) absorption in a blueberry crop during a 65-day treatment period 359 days after transplantation.

| | Coir fibre | | | Silica sand | | |
|---|---|---|---|---|---|---|
| | 0 mM | 0.6 mM | 1.2 mM | 0 mM | 0.6 mM | 1.2 mM |
| $K^+$ | 0.245c | 0.260b | 0.306a | 0.200c | 0.284a | 0.292a |
| $NO_3^-$ | 0.302c | 0.320b | 0.364a | 0.244c | 0.331b | 0.354a |
| Water | 0.322b | 0.355b | 0.431a | 0.325c | 0.384b | 0.447a |

Different letters indicate significant differences at $P < 0.05$ according to Tukey test. n= 18

**Table 2.** Vegetative growth parameters of blueberry fruits supplied with Si in the nutrient solution.

| | Coir fibre | | | Silica sand | | |
|---|---|---|---|---|---|---|
| | 0 mM | 0.6 mM | 1.2 mM | 0 mM | 0.6 mM | 1.2 mM |
| Nº leaves | 224c | 248b | 420a | 246b | 277b | 451a |
| Leaf área ($m^2$ plant$^{-1}$) | 9.18c | 10.42b | 12.03a | 9.40c | 12.37b | 14.90a |
| Leaf thickness (mm) | 0.226b | 0.243a | 0.260a | 0.220b | 0.23b | 0.271a |
| Nº stems | 14c | 20b | 24a | 11b | 19a | 22a |
| Stem diameter (mm) | 3.64b | 3.75b | 4.10a | 3.45b | 3.80b | 4.16a |
| Stem lenght (cm) | 56.24b | 57.39b | 65.87a | 59.10c | 65.18b | 70.37a |
| Leaf fresh weight (g plant$^{-1}$) | 173.43c | 202.98b | 253.07a | 180.12c | 217.10b | 260.31a |
| Leaf dry weight (g plant$^{-1}$) | 65.24c | 71.82b | 79.45a | 67.10c | 73.62b | 80.05a |
| Stem fresh weight (g plant$^{-1}$) | 109.32c | 112.99b | 152.98a | 112.61b | 116.02b | 170.53a |
| Stem dry weight (g plant$^{-1}$) | 55.6b | 59.12b | 68.10a | 59.32c | 61.03b | 69.22a |

Different letters indicate significant differences at $P \leq 0.05$ according to Tukey test. n = 18.

**Table 3.** Resistance to deformation (Shore) and breakage (N) of fruits with Si application in nutritive solution in a blueberry crop.

| | Coir fibre | | | Silica Sand | | |
|---|---|---|---|---|---|---|
| | 0 mM | 0.6 mM | 1.2 mM | 0 mM | 0.6 mM | 1.2 mM |
| Deformation | 77.3c | 84.2b | 91.1a | 78.1c | 86.3b | 90.4a |
| Breakage | 1.8c | 2.6b | 3.9a | 1.8b | 2.1b | 3.6a |

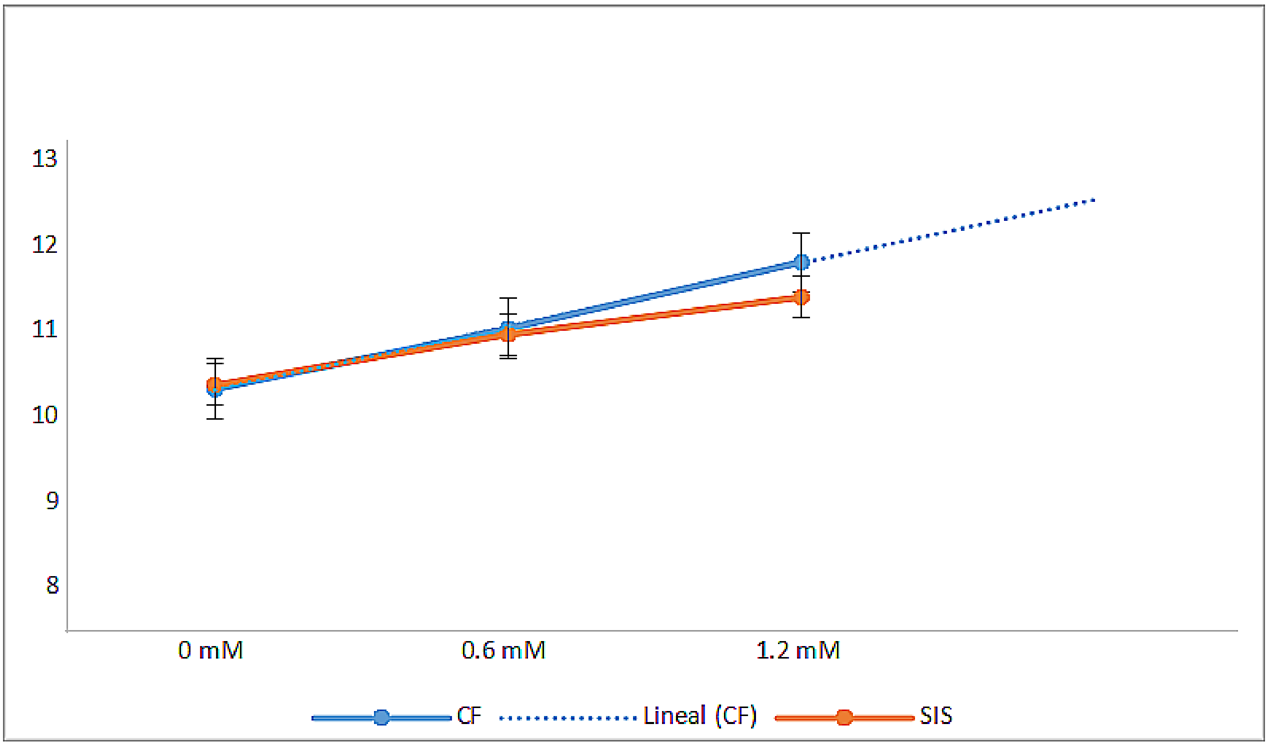
Different letters indicate significant differences at $P < 0.05$ according to Tukey test. n = 30.

**Table 4.** Blueberry fruit parameters with Si application in nutritive solution in a blueberry crop.

| | Coir fibre | | | Silicean Sand | | |
|---|---|---|---|---|---|---|
| | 0 mM | 0.6 mM | 1.2 mM | 0 mM | 0.6 mM | 1.2 mM |
| Equatorial diameter (mm) | 12.80c | 14.75b | 16.0a | 13.1b | 14.8a | 15.4a |
| Polar diameter (mm) | 9.8c | 11.43b | 13.89a | 9.4c | 11.05b | 13.10a |
| Fresh weight (g plant$^{-1}$) | 1.20b | 1.70a | 1.87a | 1.26b | 1.63b | 1.92a |
| Dry weight (g plant$^{-1}$) | 0.18c | 0.25b | 0.30a | 0.19c | 0.22b | 0.26a |

Different letters indicate significant differences at $P < 0.05$ according to Tukey test. n = 30.

Figure 1. °Brix of the fruits with the application Si in the nutritive solution in a blueberry culture. Different letters indicate significant differences at $P < 0.05$ according to Tukey test. N = 100.



# 3. Effect of ammonium nitrogen on pepper grown under soilless culture

# EFFECT OF AMMONIUM NITROGEN ON PEPPER GROWN UNDER SOILLESS CULTURE

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## Abstract

*The response of pepper (Capsicum annuum L.) to Ammonium-N was studied in a multispan greenhouse under soilless culture using coir fiber as substrate. Ammonium-N application rates were 0, 2, 4, 6 and 8 mM with a total-N concentration of 13 mM. The total content of the rest of all macronutrients in the nutrient solution was maintained from the beginning of the experiment until the end. The highest water uptake was recorded at 2 mM ammonium concentration in the nutrient solution. Compared to nitrate, ammonium nitrogen caused a decrease in nitrate absorption, but led to an increase when applying 8 mM ammonium. Despite a progressive increase in ammonium content in the nutrient solution, potassium absorption was constant below the ammonium concentration of 8 mM. Under a high N-*$NH_4$ *content (from 6 to 8 mM) in the nutrient solution, high toxicity was found, deriving in the appearance of deformed or non-commercial fruits. There was a significant benefit by the use of ammonium as a component in the nutrient solution. The optimum yield ammonium concentrations were found in the range from 2 to 4 mM. Nevertheless, concentrations higher than 6 mM led into a decrease in commercial* *production, with the production of increasing deformed fruit, out of size and/or blossom-end rot.*

**Keywords:** blossom-end rot, *Capsicum annuum,* coir substrate, hydroponics, nitrogen source, water deficit, water stress

## INTRODUCTION

Nitrogen is the mineral nutrient that plants need in the greatest quantities and the one that most frequently limits plant growth and crop yields (Forde and Clarkson, 1999; Weil et al., 2020). Nitrogen is quantitatively the main mineral element in plant tissues, and it is uptaken mostly as $NO_3^-$ or $NH_4^+$ (Miller and Cramer, 2005).

Although the highest plants can absorb amino acids through their roots as organic nitrogen source (Miller and Cramer, 2005), it is well known that inorganic nitrogen is the only nutrient taken up by plants in both an anionic ($NO_3^-$) and a cationic ($NH_4^+$) form (Forde and Clarkson, 1999).

It has been known since ancient times that growth in woody, (Bigg and Daniel, 1978; Troelstra and Blacquiere, 1986), herbaceous, (Cox and Reinsenaure, 1973; Lewis et al., 1982; Blacquière et al., 1987, 1988) or horticultural crops, (Ganmore-Neumann and Kafkafi, 1980; Claussen, 2002; Borgognone et al., 2013; Rivera-Espejel et al., 2014) using the combination of nitrate and ammonium is faster than using either one of these sources.

Despite the fact foliar application of N fertilizer had many benefits (Xu et al., 2001; Weil et al., 2020), the uncertainty of the fertilizer type, application time and usage dose exists. When ammonium is supplied to higher plants in increasing quantities, whether or not combined with another source of N (nitrate or amino acids), it is absorbed by the roots in proportion to that concentration (Wang et al., 1993). However, it is also well known that the accumulation of ammonium in any part of a plant leads immediately to the clear appearance of what (Mehrer and Mohr, 1989) called *"ammonium toxicity syndrome"*. The ammonium toxicity occurs in many natural and agricultural circumstances (Miller and Cramer, 2005), the most common causes are due to these facts: 1) problems with pH balance (Raven and Smith, 1976), 2) anion/cation imbalance (Chaillou and Lamaze, 2001) and/or 3) and the energy drain resulting from the efflux of the ion (Britto and Kronzucker, 2002).

A clear priority for plant uptake of ammonium as a source of N has been recognized to be more important than Nitrate (Souri and Hatamian, 2018); and reported that Ammonium is an attractive nitrogen form for root uptake due to its permanent availability and the reduced state of the nitrogen (Loqué and von Wiren, 2004); nevertheless when both N forms are supplied to the nutrient solution, plant root may absorb preferentially one of them, depending on the heredity of each specie (Clarkson et al. 1986; Forde and Clarkon, 1999; Akl et al., 2003; Borgognone et al., 2013), the N form supplied (Claussen, 2002), and the possible competition with other cations such as $K^{+}$, $Ca^{2+}$ y $Mg^{2+}$ (Britto and Kronzucker, 2002; Rivera-Espejel et al., 2014).

Firstly, there are the nutritive solutions for soilless cultivation systems with specifications of the Electric conductivity, pH and concentration of macronutrients and micronutrients, either with (E.g. Arnon, 1937, 1938) or without ammonium (E.g. Hoagland and Snyder, 1933; Robbins, 1946). In recent decades, numerous nutrient solutions used in soilless crops have also been published with or without ammonium in their composition depending on the cv (E.g. Sonneveld and Straver, 1994). Furthermore, in numerous cases the authors agree that the concentrations of this nutrient cation to satisfy the internal demands of the plants clearly depend on the species being cultivated (Smith et al., 1983).

Some of the general symptoms of $NH_4^+$ poisoning the plant are the leaf chlorosis and a decreased growth, which can cause a reduction in yield that can range from 15 to 60% (Gerendás et al., 1997; Britto and Kronzucker, 2002). Other symptoms are the reduced root thickness, a decreased mycorrhizal relationships, a germination and a seed set (Britto and Kronzucker, 2002).

Other studies suggest that $NH_4^+$ poisoning triggers changes in the hormonal balance between the plant and the concentration of polyamines. (Houdusse et al., 2005) have documented that ammonium nutrition induces the accumulation of mainly free putrescine, which can cause negative effects on plant development such as depolarization of membranes, leakage of $K^+$, protein losses, and tissue necrosis in wheat and pepper plants. In tomato and pepper fruits the $NO_3^-:NH_4^+$ ratio can induce the appearance of blossom-end rot and fruit deformations (Bar-Tal et al., 2001), when there is a contribution of ammonium, the absorption of K and Ca is reduced (Sarro et al., 1995). However other authors as (Houdusse et al., 2008) reported that the presence of nitrate corrected the negative effects of mixed nitrogen forms, containing ammonium and/or urea on the growth of pepper plants, but not for wheat.

Secondly, it can be admitted that there is some controversy related to the best form of N supply in the two main forms of nitric absorption: as anion or ammonium as cation that needs to be determined for each cultivar (Zandvakili et al., 2019). It is generally accepted that in soilless cultivation systems the optimal ratio can have a minimum of 75% in the nitric form versus a maximum of 25% in the cationic form (both expressed in mM concentration) (Mengel and Kirkby, 2001), however plant species differ considerably in their tolerance to $NH_4^+$. In this way, onion and corn are very tolerant, while tomato and pepper are considered sensitive crops (Gerendás et al., 1997), in spite of this, there is not much information about pepper cvs. The discrepancy in the literature may have occurred because of the fact that we need a specific information about each nutrient solution and cvs. Therefore, the form of nitrogen suitable for obtaining the maximum production for each species and its cultivation conditions has not yet been defined (Gallegos-Vázquez et al., 2000).

Consequently, work carried out with toxicity in pepper has showed that the nutrition of nitrates, ammonium and urea significantly improved the dry matter production of the shoots and roots (Houdusse et al., 2007, 2008; Garnica et al., 2012). Thus, it is suggested to associate different sources in one form of N and to correct the negative effects of ammonia.

Hence, it is sometimes difficult to distinguish clearly between osmotic general effects and specific ionic effects (Sonneveld and Straver, 2009), and it is much more difficult to differentiate between toxicity and nutrient disturbance (Bernstein, 1964).

The aim of this study was to evaluate the performance of a sensitive crop such as pepper under different concentrations of $NH_4^+$ in combination with $NO_3^-$ to identify the limit of toxicity in the plant, maintaining the content of the rest of macro and micronutrients.

## MATERIAL AND METHODS

### *Treatments and growth conditions*

The experiment was carried out at the University of Almeria (Spain), in a multi-span greenhouse whose characteristics are described by (Valera et al., 2016). Pepper plants *(Capsicum annuum* L. cv. Apolo) in the six-leaf state were transplanted on July 2, 2019 into 15 L containers of coconut fiber based on treatment with various sources of Ammonium in the nutrient solution. The composition of the nutrients in the standard nutrient solution was based as recommend by Sonneveld and straver (1994) (Treatment 0), and changing $NO_3^-$-N by $NH_4^+$-N at 2, 4, 6 and 8 mM, maintained N and the rest of macronutrients at the same level (Table 1). Fruit production was evaluated in the period from 30 to 60 days from transplantation.

### *Ammonium treatments*

The crop was fertigated with the nutritive solution. Five treatments have been considered: 0, 2, 4, 6 and 8 mM of ammonium from the same source with ammonium nitrate, with a total nitrogen proportion of 13 mM (Table 1). The pH was kept constant at 5.8 for each treatment with the addition of diluted nitric acids.

### *Fertigation parameters*

The management of the fertigation was carried out following the criteria of Urrestarazu, (2015) and Urrestarazu et al. (2017). Fertigation was proved when the 10% easy available water was consumed (Rodríguez et al., 2015). For each treatment, the drains of 4 cultivation units were collected. The pH, electrical conductivity (EC), nitrate and potassium content of the nutritive solution supplied and of the drainage were measured with pH-meter Crison MM40+ (Hach® LPV2500.98.0002, Bizkaya, Spain), a Conductivity meter EC-Meter Crison BASIC 30 (Hach® LPV2500.98.0002, Bizkaya, Spain), and LAQUATwin B-742 and B-731 (Horiba®, Northampton, UK), respectively. The volume (L) with a test tube graduated to the one hundredth of a millimeter. The absorption of water, nitrate and potassium was estimated by measuring the volume, nitrate and potassium content of nitrate in the fertigation provided and the drainage obtained (Pozo et al., 2014).

### *Yield and fruit quality*

Number, pH, total soluble solids content (TSS), Electric conductivity, length, diameter and fresh weight of fruits were measured. Non-marketable and fruit with Blossom-end rot (BER) were categorized separately. Parameters were determined in red mature fruits. TSS were determined by an Atago N1 refractometer and expressed as ° Brix at 20 ºC. The dry matter was obtained by weighing the initial fresh plant weight with an accuracy of one hundredth of a gram, and its subsequent drying in a forced air oven by subjecting the samples to 80º C to constant weight. Brix degrees were measured by refractometry, as well as pH and EC of the fruits with the same instrumentation as the drains.

### *Statistical analysis and experimental design*

The experiment was carried out under a randomized complete block design with four replications and 5 treatments (Petersen, 1994). The results were subjected to an analysis of variance (ANOVA) and subsequent mean separation using Tukey multiple range test ($P \leq 0.05$), using the Statgraphics Centurion® XVI. II. Linear and quadratic regression were used to obtain the relation between different plant parameters and ammonium concentrations.

## RESULTS AND DISCUSSION

### *Fertigation and water absorption parameters*

A significant effect of N-$NO_3^-$:N-$NH_4^+$ ratio on water absorption by the pepper crop was recorded (Figure 1). A concentration of 2 mM ammonium in the nutrient solution improved the water absorption at 10 and 6% compared to treatments 0 or 4 mM, respectively. A quadratic equation ($R^2 = 0.90$) showed that the range of 2 to 3 mM caused the best water absorption. At 8 mM of ammonium concentration a high phytotoxic effect was recorded. At a total N concentration in the nutrient solution of 7 mM (Bar-Tal et al., 2001) had also found the highest proportion of transpiration at similar N-$NO_3^-$:N-$NH_4^+$ ratio.

When no ammonium was applied there was a decrease in nitrate absorption (Figure 2). In this way, concentrations of 2, 4 and 6 mM in ammonium recorded similar nitrate absorption. Only at a concentration of 8 mM of N-$NH_4^+$ it was led a significant increase in nitrate absorption. Authors as (Bar-Tal et al., 2001) also reported progressive N-absorption from 0.25 to 14 mM of Total-N in the nutrient solution (with N-$NO_3^-$:N-$NH_4^+$ ratio of 4.0); while with an average Total-N concentration of 7 mM (with the combination of ammonium and nitrate), the N-absorption decreased as N-$NO_3^-$:N-$NH_4^+$ ratio increased above 2. On the other side, (Blacquière et al., 1988) had already reported that *Plantago lanceolata* cv had a different absorption levels depending on the nitrogen source. In *P. major* this same absorption was indifferent depending on the N supply (with nitrates, with ammonium or with the combination of both), which shows the clear importance of how the absorption is affected by the cultivar.

Forde and Clarkson (1999) reported that when $K^+$ and $NH_4^+$ share the same root ion channels, a competitive interaction between the two ions in their uptake could be expected, so it is also well known that the content in the nutrient solution of macronutrients like the ammonium cations affects the absorption of potassium (Souri and Hatamian, 2018 ). Nevertheless, the decrease of potassium absorption was not recorded until it was reached a concentration of 8 mM of ammonium in the nutrient solution (Figure 3). Bar-Tal et al. (2001), using 7 mM of total-N, found a progressive decrease of potassium absorption, while increasing relative ammonium content (N-$NO_3^-$:N-$NH_4^+$ ratio from 4 to 0.25). In contrast, in our experiment, when reaching a concentration of 13 mM of total nitrogen, it was necessary to use a level of 1.16 in the N-$NO_3^-$:N-$NH_4^+$ ratio. Consequently, this caused a decrease in potassium absorption in the presence of ammonium, suggesting that active absorption of potassium is not impaired in our cultivar until N-$NO_3^-$:N-$NH_4^+$ ratio is far less than that the found by (Bar-Tal et al., 2001).

### *Fruit growth parameters*

The best number of commercial fruits was found at the concentration of 2 mM (Table 2). At the concentration of 4 mM the same result was obtained as the control solution, and from concentrations of 6 mM or higher there was a clear decrease in the number of commercial fruits. The length of the fruit was greater when applying the lower ammonium concentration to the nutrient solution. The higher ammonium concentration registered the highest number of non-commercial fruits, these negative results from the concentration of 6 mM are produced by a considerable increase in the number of non-commercial fruits, by deformation, blossom-end Rot (BER) or by both physiopathies at the same time.

Akl et al. (2003) working in tomato grown hydroponilly (perlite substrate), with 16 mM of Total-N concentration and increasing of ammonium to values above 0.10 mM (N-$NO_3^-$:N-$NH_4^+$ ratio), restricted progressively the vegetative growth, the fruit yield and quality, and enhanced markedly the incidence of blossom-end rot. Contrary to this, our results showed that the ratio value of 1.50 (6 mM de ammonium) for pepper fruits to deform or BER to appear was significant. These results suggest that cv. pepper is more tolerant to higher ammonium concentration than tomato.

The increase of BER could be a consequence of critical levels and competitive restriction of Ca (and Mg) uptake by ammonium (Akl et al., 2003). No clear results and conclusions were reached on the quality parameters (TSS, pH and EC) of the evaluated fruits. These results agree with the publications of multiple authors who recommend to apply ammonium in the nutritive solution until 2.5 mM and not to exceed these limits that have been collected in the introduction.

### *Production*

Figure 5 and 6 show the commercial and total production of pepper fruits. The effect of ammonium concentration was clear and significant. In both cases a clear benefit was found with the application of ammonium to the nutrient solution with a very high quadratic coefficient of significance ($R^2 > 0.99$).

More than 10% of commercial production was obtained by applying 2 mM to the nutritive solution. The concentration of 2 to 4 mM obtained the best results in the total production with a value higher than 10% with respect to the control (0 mM of ammonia) or to the dose of 6 mM. The difference in the optimum between 2 and 4 mM of ammonium to recommend, for a commercial production (optimum in 2 mM) and a total (optimum plus between 3-4 mM) is due to the increase of fruits that do not become commercial (Table 2).

It was necessary to reach a concentration of 8 and 6 mM (equivalent to $N\text{-}NO_3^-:N\text{-}NH_4^+$ ratio = 1.16 and 1.50) to find a decrease the commercial and non-commercial yield, respectively. In contrast, a $N\text{-}NO_3^-:N\text{-}NH_4^+$ ratio = 4, has been related to reduced fruits of pepper (Zornoza et al., 1987; Bar-Tal et al., 2001).

Also Claussen (2002) under sand culture found an increase in fruit dry weight (11% and 30%), if supplied in addition to nitrate nitrogen with ammonium at same ratio from 3 to 1, whilst (Akl et al., 2003) found the best production in the ratio between 0.05 and 0.15.

## CONCLUSIONS

An application of 2 mM of ammonium to the nutritive solution generates a benefit close to or higher than 10% in pepper cultivation, both in the cultivation parameters and in the production in commercial weight and number of fruits. The range of 1.5 to 3.0 mM of ammonium produces a higher and better commercial yield than the option of not applying ammonium to the nutrient solution. When 4 mM ammonium in the nutrient solution is reached, production is similar to the treatment without application of ammonium. In order to find ammonium toxicity levels in the case of pepper cultivation, the value of 4 mM in commercial production and 6 mM in total production must be clearly exceeded.

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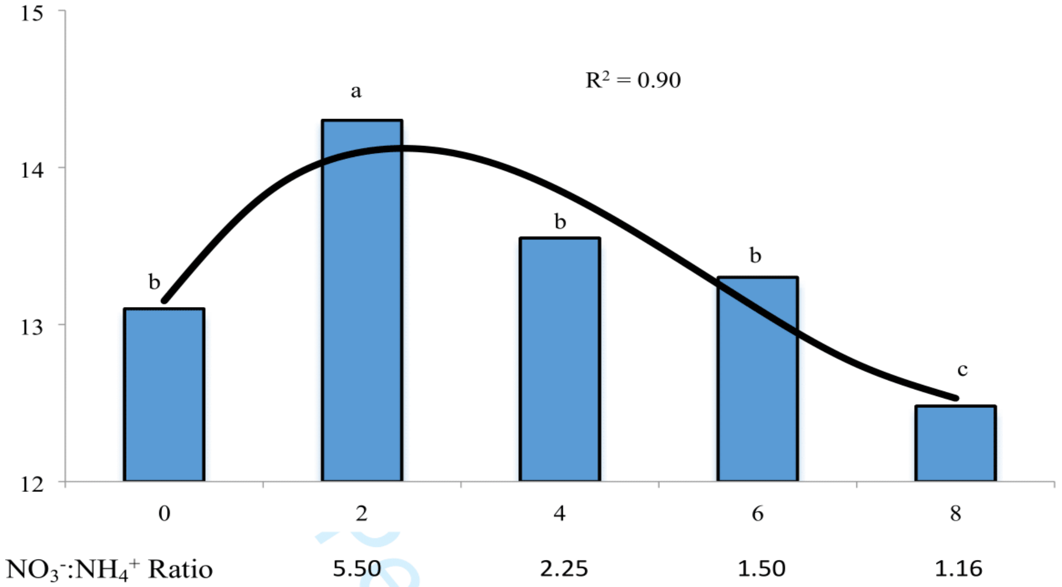

**FIGURE 1.** Water absorption (L plant$^{-1}$) as a function of the concentration in mM of ammonium in the nutrient solution in a pepper crop during 130 days after transplant. Means with a different letter indicate a significant statistical difference according to the Tukey test ($P \leq 0.05$).

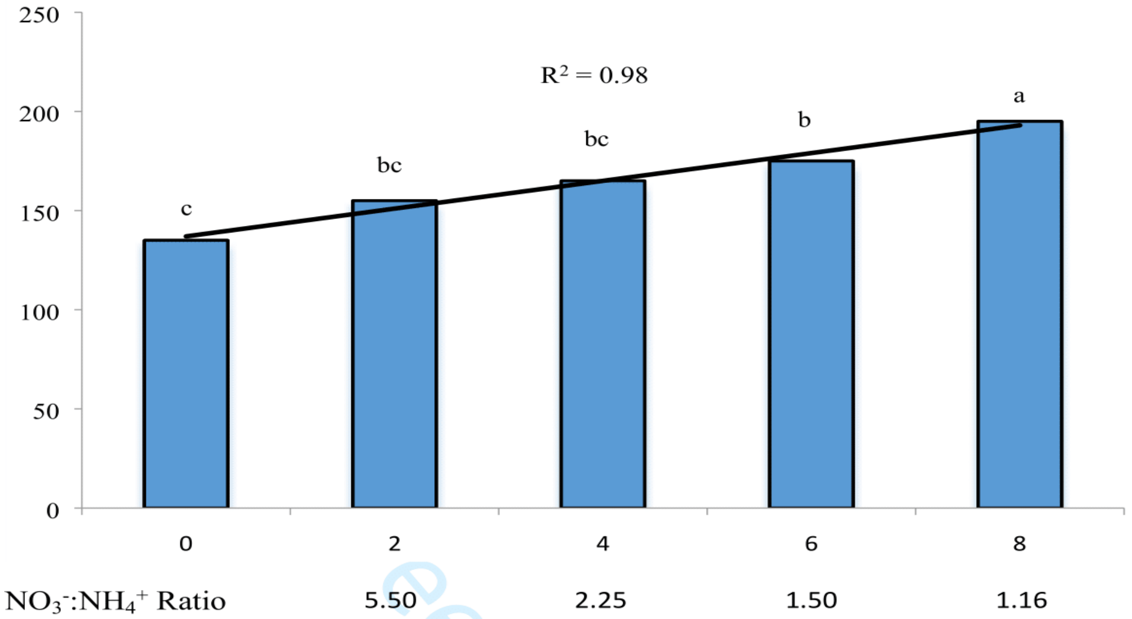

**FIGURE 2.** Nitrate absorption (mmol plant$^{-1}$) as a function of the concentration in mM of ammonium in the nutrient solution in a pepper crop during 130 days after transplant. Means with a different letter indicate a significant statistical difference according to the Tukey test ($P \leq 0.05$).

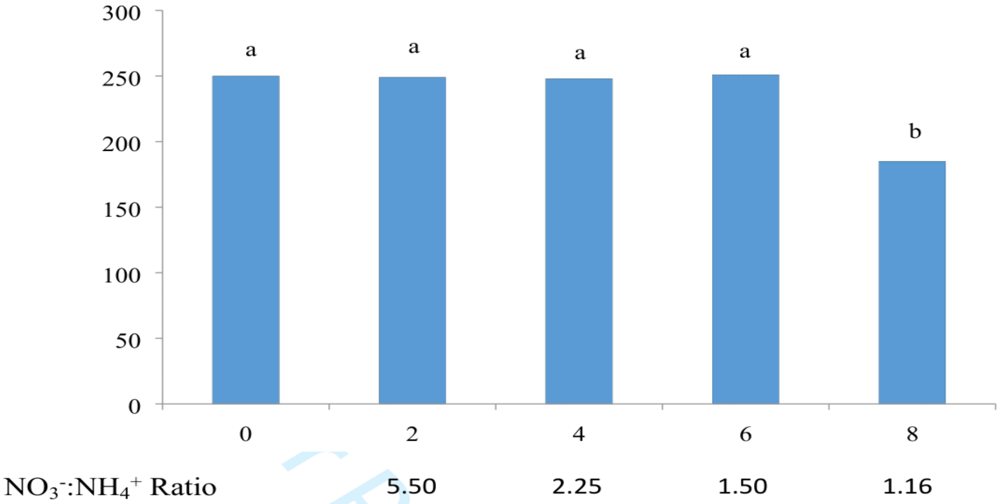

**FIGURE 3.** Potassium absorption (mmol plant$^{-1}$) as a function of the concentration in mM of ammonium in the nutrient solution in a pepper crop during 130 days after transplant.

Means with a different letter indicate a significant statistical difference according to the Tukey test ($P \leq 0.05$).

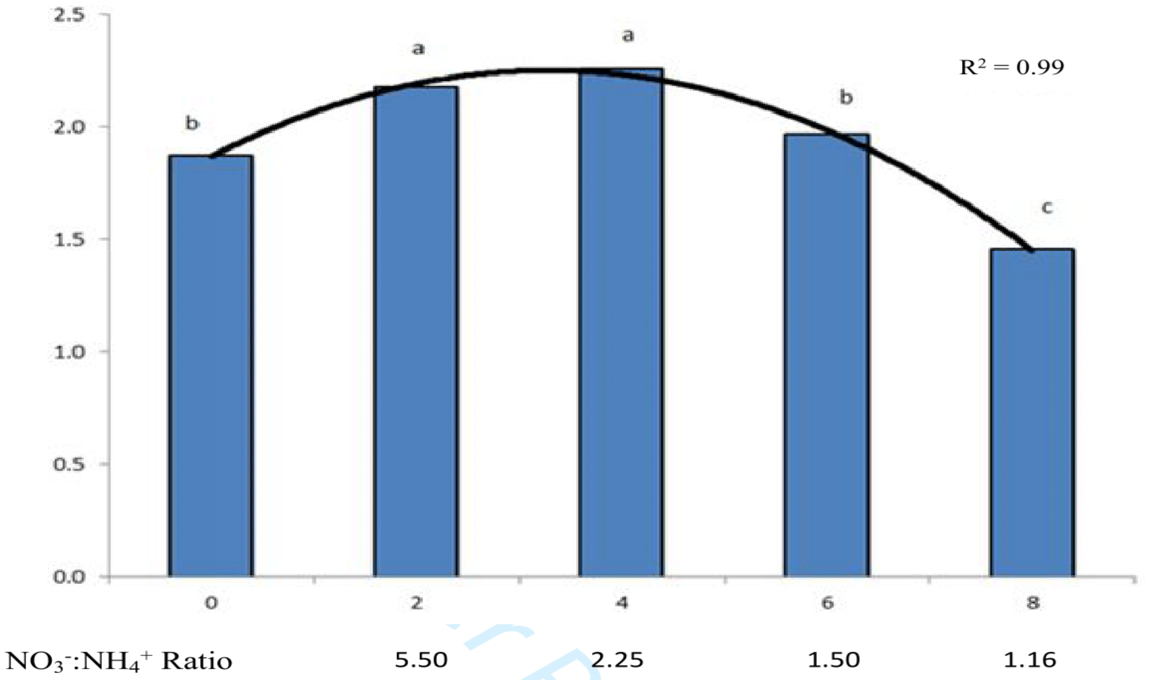

**FIGURE 4.** Total Yield (kg plant$^{-1}$) as a function of the concentration in mM of ammonium in the nutrient solution in a pepper crop during 130 days after transplant. Means with a different letter indicate a significant statistical difference according to the Tukey test ($P < 0.05$).

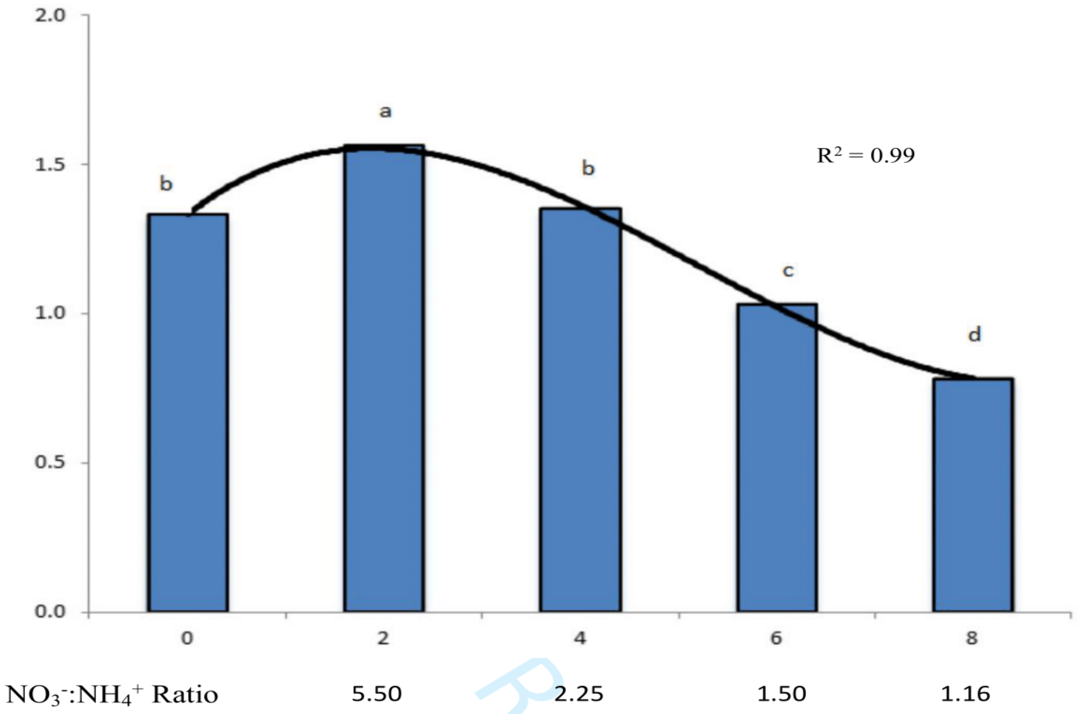

**FIGURE 5.** Commercial Yield (kg plant$^{-1}$) as a function of the concentration in mM of ammonium in the nutrient solution in a pepper crop during 130 days after transplant. Means with a different letter indicate a significant statistical difference according to the Tukey test ($P < 0.05$).

**TABLE 1** Anion content (mN) by commercial fertilizers applied in the experiment and the values of different source of Nitrogen

| Treatment | | $H^+$ | $K^+$ | $Ca^{2+}$ | $Mg^{2+}$ | $NH_4^+$ | N $NO_3^-$ | N $NH_4^+$ | Total -N | $NO_3^-$:$NH_4^+$ |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | $NO_3^-$ | 1.00 | 5.00 | 5.00 | 2.00 | 0.00 | 13.00 | 0.00 | 13.00 | |
| | $PO_4H_2^-$ | 2.50 | | | | | | | | |
| | $SO_4^{2-}$ | 2.00 | | | 0.50 | | | | | |
| | $Cl^-$ | | | | | | | | | |
| 2 | $NO_3^-$ | | 4.00 | 5.00 | | 2.00 | 11.00 | 2.00 | 13.00 | 5.50 |
| | $PO_4H_2^-$ | 2.50 | | | | | | | | |
| | $SO_4^{2-}$ | | | | 2.50 | | | | | |
| | $Cl^-$ | | 1.00 | | | | | | | |
| 4 | $NO_3^-$ | | 0.00 | 5.00 | | 4.00 | 9.00 | 4.00 | 13.00 | 2.25 |
| | $PO_4H_2^-$ | 0.50 | 2.00 | | | | | | | |
| | $SO_4^{2-}$ | | | | 2.50 | | | | | |
| | $Cl^-$ | | 3.00 | | | | | | | |
| 6 | $NO_3^-$ | | | 5.00 | | 4.00 | 9.00 | 6.00 | 13.00 | 1.50 |
| | $PO_4H_2^-$ | | 1.00 | | | 1.50 | | | | |
| | $SO_4^{2-}$ | | | | 2.00 | 0.50 | | | | |
| | $Cl^-$ | | 4.00 | | | | | | | |
| 8 | $NO_3^-$ | | | 5.00 | | 4.25 | 9.25 | 8.00 | 13.50 | 1.16 |
| | $PO_4H_2^-$ | | 0.50 | | | 2.00 | | | | |
| | $SO_4^{2-}$ | | | | 1.75 | 1.75 | | | | |
| | $Cl^-$ | | 4.50 | | | | | | | |

**TABLE 2** Effect of $NH_4^+$-N[1] content in nutrient solution on marketable yield, total soluble solids content (TSS), pH, electric conductivity (EC) and occurrence of deformed and blossom-en rot (BER) fruit in pepper grown in coir fiber substrate

|  | Commercial |  |  |  |  |  | Non-commercial |  |  |
|---|---|---|---|---|---|---|---|---|---|
| mM | Fruit No. | Fruit length (cm) | Fruit diameter (cm) | TSS ° Brix | pH | EC (dS $m^{-1}$) | Fruit No. | Deformed% | BER% |
| 0 | 19.16b | 6.46a | 5.94ab | 6.91b | 4.42a | 5.31a | 4.32e | 0.05c | 0.00c |
| 2 | 25.15a | 6.62a | 6.41a | 7.71a | 4.49a | 4.86ab | 5.21d | 2.00b | 0.17c |
| 4 | 20.34b | 6.22ab | 5.84b | 7.56a | 4.58a | 5.06ab | 8.01c | 2.50ba | 2.57b |
| 6 | 15.82c | 5.90b | 5.46bc | 6.82b | 4.50a | 4.73b | 11.32b | 3.01a | 4.45a |
| 8 | 17.49c | 5.78b | 5.06c | 7.94a | 4.48a | 4.68b | 12.82a | 3.01a | 5.09a |

Different letters indicate significant differences at $P \leq 0.05$ in a Tukey Test.

[1]: Total-N=13 mM.

# 4. LED enhances plant performance and both carotenoids and nitrates profiles in lettuce

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ORIGINAL PAPER

# LED Enhances Plant Performance and Both Carotenoids and Nitrates Profiles in Lettuce

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## Abstract

Recent studies show that vegetables at early stage of development contain higher amounts of phytonutrients and minerals, and lower amounts of nitrates than at fully developed stage. Nevertheless, the effects of some spectrum light on the carotenoid content of lettuce microgreens are unknown. Three different LED lamps were checked: (i) artificial white light ($T_0$); (ii) continuous light-emitting diodes with longer blue-wavelength ($T_1$), and (iii) continuous light-emitting diodes with longer red-wavelength ($T_2$). Different lettuce cvs. were grown under the above described lamps. Plants were collected after 10, 15, 35 and 50 days from planting to produce sprouts, microgreens, initial baby leaf, and baby leaf, respectively. Response to different continuous spectrum lights related to productivity and nitrate content was variable for the different plants. Accumulation of nitrates at initial stages in plant tissues was clearly lower than at final stages of crop development, ranging from 50.2 to 73.4 mg 100 $g^{1}$ fresh weight for $T_2$. Lettuce consumption is preferable at microgreen stage in comparison with baby leaf stage. Nitrate amounts at microgreen stage were lower than in baby leaf stage, and this content was inversely correlated with carotenoid content, which in tissues was higher at microgreens stage influenced by LED.

**Keywords** *Lactuca sativa* · Continuous light spectrum · Microgreens · Baby leaf · Sprouts

## Introduction

Microgreens are a new trend for consumption of vegetables, which are harvested with well-expanded cotyledon leaves and the presence or absence of the first pair of true leaves [1]. Although they are species-dependent, harvesting takes place after approximately 7–21 days from germination and at ground level, so that the edible portion includes the hypocotils [2, 3]. Different studies have shown that microgreens may contain higher amounts of phytonutrients (ascorbic acid, $\beta$-carotene, phylloquinone, $\alpha$-tocopherols), minerals and lower figures of nitrates as compared to mature stage vegetables [4–6]. The *Lactuca sativa* L. cv. contains significant cocentrations of carotenoids at the microgreen stage. Also, Brassicaceae microgreens such as broccoli, cabbage and radish, contain high amounts of glycosilonates, which are reported as antitumor compounds. However, evidence exits on that nitrates react with other secondary amines to produce nitrosamines, which induces gastrointestinal cancer. Therefore, nitrate contents of vegetables need to be controlled. Such content depend on several factors: (i) cv. variety [7, 8], (ii) growing season, (iii) the wavelength received by the plant, (iv) the culture system, and (v) fertigation [7]. To avoid the drawback caused by the consumption of lettuce, the; European Union regulated in 2011 the maximum allowable concentration: 500 and 400 mg $NO_3^-$ 100 $g^{-1}$ fresh weight (fw) for lettuces harvested in winter and spring, respectively; and 250 mg $NO_3^-$ 100 $g^{-1}$ f w for iceberg cv [9].

Lighting have strong influence in the morphology of microgreens and in the biosynthesis and accumulation of chemical compounds, especially under controlled growth conditions [1]. LED lighting systems have important advantages over traditional lightings due to their spectral composition, durability, specificity of wavelength, low radiant heat and energy efficiency [10]. The effects of the quality of the light spectrum on the production features and the influence on bioactive compounds contents in vegetables are well-known [10–13].

Although the influence of irradiation dose on the carotenoid content of baby leaf is known [4], while knowledge on

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nitrate content of microgreens at mature stages is undeveloped [6], nor the effects of lighting spectrum on such content [14]. Consistently, the objective of this work was to check the influence of the light spectrum type on carotenoids and nitrates contents on lettuce harvested at different development stages.

## Materials and Methods

The experiment was carried out at the University of Almería (Spain), in a controlled growth chamber (10 m x 2.5 m) between 2019 and 2020. LED treatments have been used to evaluate the quality of different lettuce cv. Plants were exposed to different treatments with LED lamps: linear spectrum ($T_0$) and two common continuous spectrum lamps: ($T_1$), with a photonic flow of 141 $\mu$mol $m^{-2}$ $s^{-1}$ and illumination of light emitting diodes of 5161 lx; and ($T_2$), with a photonic flow of 107 $\mu$mol $m^{-2}$ $s^{-1}$ and illumination of light emitting diodes of 3837 lx, respectively. White LED lamps L 18 T8 Roblan® (Toledo, Spain) were used as control ($T_0$), L 18 NS1 Valoya® (Helsinki, Finland) as treatment ($T_1$), continuous light-emitting diodes with a longer wavelength in blue, which is responsible for vegetative growth but with less energy than red light[22] and L18 AP67 Valoya® (Helsinki, Finland) as treatment ($T_2$) a continuous light-emitting diodes with a longer wavelength in red. The technical specifications are shown in Table 1. For the determination of the spectral characteristics, six measurements were made at 20 cm from the panel in which LED tubes were housed. An HD 2302.0 photoradiometer (Delta OHM®, Veneto, Italy) was used to measure quantitative light. The LP 471 PAR and LP 471 PHOT probes were used to measure photosynthetic photon flux density (PPFD, µmol $m^{-2}$ $s^{-1}$) and luminance (lux), respectively. The spectra of the treatments $T_0$, $T_1$ and $T_2$ were recorded with the UPRtek MK350S spectrometer (Miaoli, Taiwan) and are shown in Fig. 1. The photoperiod was 16/8 h (day/night) at 20 °C and 80–85 % relative humidity.

Three different lettuce cv were used: *L. sativa* cvs. Romana, Angel and Mantecosa. Plants were collected at four different development stages: sprouts (0–1 true leaves), microgreens (2–3 leaves), initial baby leaf (7–8 true leaves) and baby leaf (16–17 leaves), after 10, 15, 35 and 50 days from sowing, respectively. After harvesting, plants were stored in thermal bags and frozen at -24 ºC. For sprouts and microgreens, 3.3 g of seeds *per* tray (43.5 cm x 28 cm) filled with coconut fibre were used (four replicates for each developmental stage). For the initial baby leaf, 1 L pots were used, filled with coconut fibre. Plants were fertilized with a standard nutrient solution [15], at pH 5.8 and EC 2.2 dS $m^{-1}$. Physical characteristics of the coconut fibre used as substrate have been previously reported [16].

**Table 1** Photosynthetic photon flux density (PPFD, $\mu$mol·$m^{-2}$ $s^{-1}$) and illuminance (lux) of light-emitting diodes (LEDs) of different spectra lamps used in horticulture

| Light treatment | LED lamp type | PPFD | Illuminance |
|---|---|---|---|
| $T_0$ | L18 T8 Roblan | 150±7a | 6447±186a |
| $T_1$ | L18 NS1 Valoya | 141±19b | 5161±229b |
| $T_2$ | L18 AP67 Valoya | 107±7c | 3837±358c |

Different letters indicate significant differences at $P \leq 0.05$ by Tukey test ($n = 6$)

### Determination of Nitrate Content

Romana cv. was analyzed at the developmental stage of sprouts, microgreens, initial baby leaf and baby leaf lettuce, while the other cv. only were checked at microgreens and baby leaf stages. This was performed to acquire the highest contrast between values according to development stages. The juice was extracted by grinding plants, and then the juice was stored in 2 mL microtubes at 20–22 °C. After analyses, adequate aliquots of the juice were exposed to the device sensor. Nitrate content was measured using a LAQUAtwin $NO_3^-$ device (HORIBA®, Kyoto, Japan). Samples were measured 9 times, and averaged.

Carotenoids were extracted following the method of Kimura et al. [17]. Firstly, 5 g of freshly ground sample and 10 mL of diethyl ether was kept in darkness for 1 h and shaking it for 30 s every 5 min. Secondly, separation of the supernatant from the solid and repetition of the process until all the stain is removed from the sample. A total of six extractions were performed until all the sample was extracted. Subsequently, samples were saponified with MeOH:KOH (9:1, *v/w*). Later, the formed soaps were removed with water by decanting the aqueous phase through a decanting funnel. The solvent was evaporated from the ethereal phase under vacuum with a rotary evaporator and then extracted using a solution of diethyl ether. Subsequently, the extract obtained was filtered. Finally, carotenoid extracts were analyzed using an HPLC system.

HPLC analyses were performed in a Finningan Surveyor Chromatograph (Thermo Electron, Cambridge, UK), equipped with a UV detector and a reversed phase column (Hypersil Gold, 250 4.6 mm i.d., 5 ym particle size) (Thermo Electron, Cambridge, UK). A binary system of solvent in isocratic elution was used (acetonitrile:MeOH, 85:15, v/v). The injection volume was 5 $\mu$L, the mobile phase flow was 1 mL $min^{-1}$, the column temperature was 32 ºC and the detection wavelength was λ = 476 nm. The identification of the main carotenoids was made based on their characteristic retention times, established by using commercial standards. Carotenoids were quantified using, $\beta$-carotene (Sigma Aldrich (St. Louis, MO, USA) as an external standard, and through response factors, response factor of each carotenoid

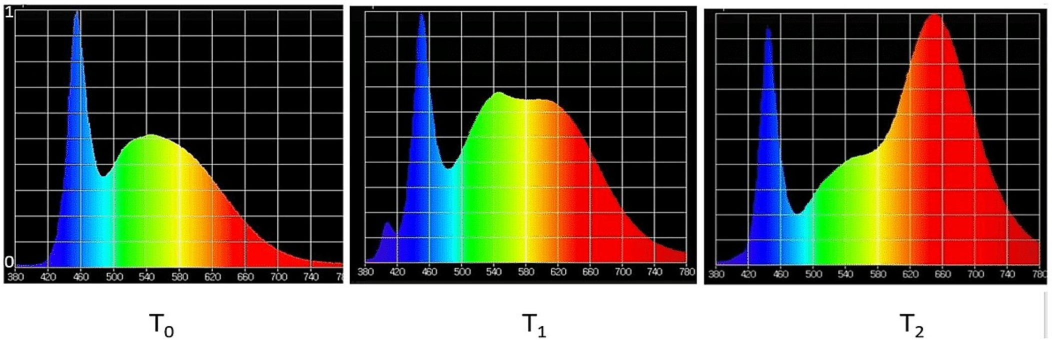


**Fig. 1** Photon flux distributions in the 380 to 780 nm spectrum for treatments. $T_0$ = L18 T8 Roblan®, $T_1$ = L18 NS1 Valoya®, $T_2$ = L18 AP67 Valoya®

relative to the reference carotenoid. These were calculated according to Kimura et al. [18].

The experimental design was in complete random blocks with eight repetitions for four treatments and three treatments. Vegetative analysis data was subjected to an analysis of variance and their averages were compared with an ANOVA test Tukey test using Statgraphics Centurion® 18.

## Results and Discussion

### Vegetative Growth

Figure 2 shows the fresh weight and height of lettuce growth in relation to leaves number. Lettuce weight showed a polynomial curvilinear growth with a very significant correlation ($R^2 = 0.82$) superior to a linear adjustment ($R^2 = 0.64$), and in the initial phase, changing from the initial microgreen stage to the sprouts stage, showed an exponential growth tendency, where the f w increased by 122 %. In the following stages f w changed between 12 and 10 % from initial baby leaf sprout stage to baby leaf stage. The sprout, microgreens, initial baby leaf and baby leaf were obtained at 10, 15, 35 and 50 days, respectively. Height had also a polynomial curvilinear growth but with a correlation ($R^2 = 0.94$) similar to that the linear adjustment ($R^2 = 0.84$), while the increasing of leaves number was adjusted to a sigmoid curve. There was a lower leaf development between the microgreens and shoots phase, with a significant difference of fresh weigh about 14 % ($P \leq 0.05$). Nevertheless, between the sprouts and initial baby leaf stages there was a very significant increase of 350 % ($P \leq 0.05$), moreover for the next stage (baby leaf) there was also a significant increase, although much lower (12 %).

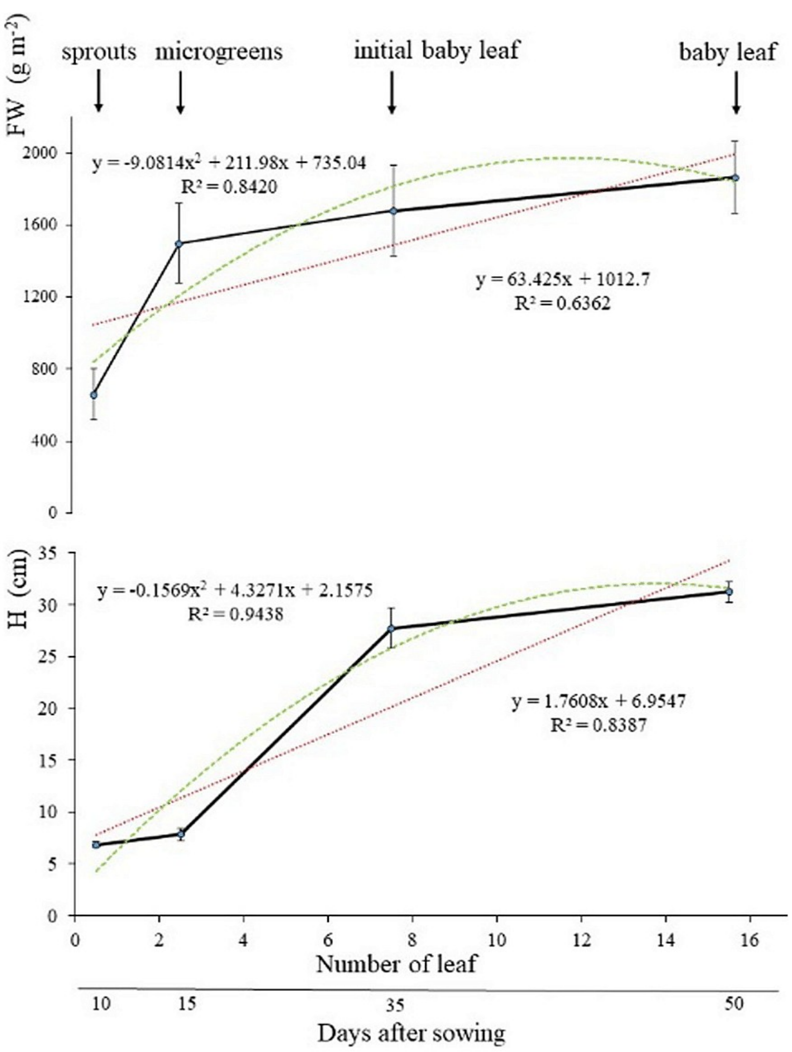
The effect of the three light spectra on the vegetative growth was variable, but in no case the white light ($T_0$) had a better performance than the commercial lighting designed for use in artificial lighting in ($T_1$ and $T_2$) (Table 2). The $T_2$ spectrum induced higher growth rate to all cv. than $T_0$, and Mantecosa cv. recorded the higher growth rate (1211 g $m^{-2}$) and plant height (7.28 cm), with a significant increase of 51 and 40 %, respectively. This was due to that using red and blue lights ($T_2$) plant growth significantly increased as compared with white fluorescent lamps ($T_0$) or blue LED ($T_1$) applying the light treatments in the same timeframe [19].

These results agree with those of other authors [10], who obtained 10.43 $g^{-1}$ lettuce plant f w using the same lighting (T2) treatment in, comparing to 5.20 g $plant^{-1}$ total f w by applying T0; while other authors [20] found a better growth on lettuce using lighting treatments with wavelengths similar to T1 and T2 than using white light T0. In radicchio and lettuce recorded a significant increase in fresh biomass using LED with prevalent red radiation compared to white fluorescent lamps. Besides red and blue spectrum, there is a continuous spectrum close to the ideal of photosynthesis, which not only increased the higher productivity of lettuce, but also substantially reduced the nitrate content [10].

### Nitrate Content

Nitrate contents are summarized in Tables 3 and 4. All nitrate values were within the limits allowed by the European Union regulations [9]. Amounts ranged from 80 % of the stablished lower limit (for microgreens and shoots) to 50–70 % for the sam limit in the case of initial baby leaf and baby leaf, respectively. $T_0$ in all cv. induced the higher content nitrate values, except $T_2$, which registered the lowest values for all lettuce cv. Such values were also lower than other average data published by the European Food Safety Authority (EFSA) [21] (200 to 400 mg 100 $g^{-1}$ f w) and US average values of other authors [22] (135.6 to 398.3 mg 100 $g^{-1}$ f w), in comparison to data obtained in this experiment for baby leaf (71.7 to 132.5 mg 100 $g^{-1}$ f w). In both cases on lettuce experiments, LED treatments were not applied. Conversely, other authors [23] indicated values were similar to those obtained in this study, showing nitrate levels of 16 and 60 % lower than the legal higher limits recorded [9].

**Fig. 2** Weight (FW) and height (H) of lettuce depending on the development stage ($n = 9$)



A significant variation on nitrate content was recorded in the different stages of development. For instance, in cv. Romana cultured using $T_0$ lamps, in the change from microgreens to the initial baby leaf stage, the nitrate content significantly increased a 36 % higher in comparison with amounts detected in sprouts and microgreens. This was

**Table 2** Microgreen fresh weight (FW, g m$^{-2}$) and height (H, cm) of three lettuce cvs. grown under different light-emitting diode (LED) lamp spectra

| | FW | | | H | | |
|---|---|---|---|---|---|---|
| | Mantecosa | Angel | Romana | Mantecosa | Angel | Romana |
| $T_0$ | 800±18.9bA | 529±12.8bB | 914±12.3aA | 5.12±0.1cB | 3.30±0.2bC | 6.52±0.7bA |
| $T_1$ | 1090±23.1aA | 593±9.34bB | 1060±8.2aA | 6.26±0.4bA | 3.49±0.5bB | 6.75±0.3abA |
| $T_2$ | 1211±10.7aA | 694±20.6aC | 892±10.4aB | 7.28±0.8aA | 4.42±0.2aB | 7.21±0.4aA |

$T_0$ = L18 T8, $T_1$ = L18 NS1, $T_2$ = L18 AP67. Lowercase and uppercase different letters in the same column and row indicate significant differences to light treatments and stage of crop at $P \leq 0.05$ by Tukey test, respectively ($n = 9$)

recorded in the baby leaf phase, which reached 132.5 mg$^{-1}$ 100 g$^{-1}$ f w, while the lowest value of nitrates was recorded with $T_2$ (50.2 mg$^{-1}$ 100 g$^{-1}$ f w). Other authors [6] evaluated lettuces in greenhouses with natural lighting and found that the nitrate content in baby leaf lettuce were 80 % lower than in mature stages. This was due to that in the experiments the authors used a light intensity ranging between 3790 and 4920 lx, while in our experiment $T_0$ had 6447 lx. Furthermore, different light intensities have a significant effect on nitrate content [24]. Pinto et al. [6] obtained for $T_2$ similar results to those used in our experiment, and using also a similar lighting control ($T_0$ treatment). The light intensity was one of the major factors influencing nitrate content in leafy vegetables [25]. Other authors [26] reached in microgreens nitrate values of 126 mg 100 g$^{1}$ without LED treatment application.

White LED light ($T_0$) always induced a significant and higher concentration of nitrates (20 %) than values obtained using the $T_1$ and $T_2$ spectra in both microgreens and baby leaf stage. In microgreens cv. Angel, $T_2$ spectrum induced the better response to reduced nitrate content. The only cases in which $T_1$ spectrum induced higher nitrate content (59.2 mg 100 g$^{-1}$ f w) than other lighting treatments were in cv. Mantecosa and cv. Romana (54.1 mg 100 g$^{-1}$ f w). These values are acceptable for human health according to established limits for nitrate in green vegetables [9]. These results of the present work match with values from other authors [14, 27], who showed that in lettuce and spinach nitrate content was significantly lower in treatments with red and blue light or a mixture of both lights in comparison to white light. This may be due to the activity of the enzyme nitrate reductase, as stated by other authors [28], who found a decrease in nitrate levels using red light. Similar results were also obtained by other authors [20] in lettuce sprouts. All these results are probably influenced by the fact that red and blue lights promote growth and the level of carbohydrates in plants [29].

## Carotenoid Content

Carotenoids content are summarized in Table 5, and a chromatogram of a microgreen sample is shown in Fig. 3. Carotenoid profiles were determined only at microgreens and baby leaf stages, because lettuce at these stages shows higher significant responses to nitrate content, registering the lowest and highest amounts, respectively [4–6]. Lettuce microgreens had increased contents of carotenoids as compared to mature stages. $T_2$ lamps induced higher concentration of carotenoids (11.5 %) than that obtained by using $T_1$ and $T_0$ spectra ($P \leq 0.05$). This tendency matches with research done on the effects of LED treatments on microgreens [11]. Furthermore, these data agree with previous research on that $T_0$ treatment induces lower carotenoids amounts in microgreens than using other light spectra [4, 11].

**Table 3** Nitrate content (mg 100 g$^{-1}$ fresh weight) of Romana cv. lettuce grown under different treatments of light-emitting diode (LED) lamp spectra

| | Sprouts | Microgreens | Initial baby leaf | Baby leaf |
|---|---|---|---|---|
| $T_0$ | 89.43±0.95aD | 93.01±0.62aC | 125.02±1.28aB | 132.50±1.14aA |
| $T_1$ | 52.82±0.13bD | 59.13±0.51abC | 66.23±0.57bB | 78.32±0.39bA |
| $T_2$ | 50.24±0.25cD | 53.22±0.47bC | 63.14±0.94bB | 71.71±0.71cA |

$T_0$ = L18 T8, $T_1$ = L18 NS1, $T_2$ = L18 AP67. Lowercase and uppercase different letters in the same column and row indicate significant differences to light treatments and stage of crop at $P \leq 0.05$ by Tukey test, respectively ($n = 9$)

Zeaxanthin amounts in microgreens cv. Angel presented the lower zeaxanthin values, with 17 μg 100 g$^{-1}$ using blue-$T_1$, and 112 μg 100 g$^{-1}$ f w. using red-$T_2$. Mantecosa cv. was affected in the same way: $T_1$ and $T_2$ induced 121 and 87 μg 100 g$^{-1}$ f w. The higher values were found in cv. Romana, with 232 μg 100 g$^{-1}$ f w using $T_2$. The last cv. increased zeaxanthine amounts at microgreen stage: 123 (blue lighting-$T_1$) and 109 μg 100 g$^{-1}$ f w (white lighting-$T_0$). The influence of blue light dosage on total carotenoid content in microgreens has been previously cited [12]. β-carotene was the main carotenoid in all samples of microgreens. The content of β-carotene in $T_2$-treated cv. Angel was 4500 μg 100 g$^{-1}$ f w, higher than that obtained using $T_1$ (4168 μg 100 g$^{-1}$ f w). $T_2$-treated Mantecosa cv. showed the higher content of β-carotene with 4647 100 μg g$^{-1}$ f w, and 3636 μg 100 g$^{-1}$ f wt using $T_0$. The higher amounts of lutein in microgreens were recorded in cv. Mantecosa with $T_2$ treatment (4261 μg 100 g$^{-1}$ f w). Lutein content increased when blue and red light treatments ($T_1$ and $T_2$) were applied to microgreen lettuce cv. Romana, reaching 3093 μg 100 g$^{-1}$ f w and 3039 μg 100 g$^{-1}$ f w, respectively. The red light (330 μmol m$^{-2}$ s$^{-1}$ PPFD irradiance) stimulates lutein synthesis in Brassicaceae microgreens [11]. This agree with our results: $T_2$ induced

**Table 4** Nitrate content (mg 100 g$^{-1}$ fresh weight) of three lettuce cultivars grown under different light-emitting diode LED lamp spectra

| Stage | Light | Mantecosa | Angel | Romana |
|---|---|---|---|---|
| Microgreens | $T_0$ | 79.12±1.29aB | 70.12±1.12aC | 89.40±1.51aA |
| | $T_1$ | 59.23±0.37bAB | 61.83±0.95bA | 52.83±0.57bB |
| | $T_2$ | 73.41±0.64abA | 54.13±0.83cB | 50.24±0.79bB |
| Baby leaf | $T_0$ | 100.81±2.01aB | 93.23±1.71aC | 132.56±2.27aA |
| | $T_1$ | 80.30±0.94cA | 82.32±0.62bA | 78.35±0.79bA |
| | $T_2$ | 92.53±1.06bA | 78.75±1.14bB | 71.74±0.35cB |

$T_0$ = L18 T8, $T_1$ = L18 NS1, $T_2$ = L18 AP67. Lowercase and uppercase different letters in the same column and row indicate differences to light treatments and cvs at $P \leq 0.05$ by Tukey, respectively ($n = 9$)

**Table 5** Carotenoid content in microgreens and baby leaf ($\mu$g 100 g$^{-1}$ fresh weight) of three lettuce cultivars grown under different light-emitting diode (LED) lamp spectra

| | $T_0$ | | | $T_1$ | | | $T_2$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mantecosa | Angel | Romana | Mantecosa | Angel | Romana | Mantecosa | Angel | Romana |
| Microgreens | | | | | | | | | |
| $\beta$-carotene | 2941±190aB | 2669±23bC | 2271±22cC | 4591±61aA | 4168±16bB | 3679±109cB | 4647±363bA | 4500±407bA | 5836±642aA |
| Lutein | 2270±167aC | 237±5cC | 955±25bB | 2571±633bB | 391±37cB | 3093±326aA | 4261±29aA | 1240±242cA | 3039±235bA |
| Zeaxanthin | traces | traces | 109±42aC | 87 ± 6a | 17 ± 1bB | 123±17aB | 121 ± 20b | 112 ± 16bA | 232 ± 12aA |
| Total | 5211 C | 2906 C | 3335 C | 7249B | 4576B | 6895B | 9029 A | 5852 A | 9107 A |
| Baby leaf | | | | | | | | | |
| $\beta$-carotene | 532±99aC | 215±15cC | 400±33bC | 930±136aB | 578±104bB | 603±67bB | 2103±190aA | 1436±135bA | 706 ± 99cA |
| Lutein | 836±111abC | 622±176bB | 919±113aC | 1172±41aB | 630±22bB | 1192±18aB | 1373±87aA | 811±52bA | 1432±82aA |
| Zeaxanthin | 57±20bB | 61±29bA | 90±53aA | 74±65aA | 10±5bB | traces | 79±28aA | traces | 75±18aA |
| Total | 1425 C | 452 C | 1409 C | 2176B | 1218B | 1795B | 3555 A | 2247 A | 2213 A |

$T_0$ = L18 T8 Roblan, $T_1$ = L18 NS1 Valoya, $T_2$ = L18 AP67 Valoya. Lowercase and uppercase different letters indicate differences in cvs. and light treatments at $P \leq 0.05$ by Tukey, respectively ($n = 6$)

1240 µg 100 g$^{-1}$ f w lutein in cv. Angel., while by using $T_0$ and $T_1$ 237 and 391 $\mu$g 100 g$^{-1}$ f w were obtained.

At baby leaf stage, cv. Mantecosa showed the higher amounts of $\beta$-carotene in comparison with the other two cv. For $T_2$, cv. Mantecosa registered 2103 $\mu$g 100 g$^{-1}$ f w, while by using $T_1$ 930 $\mu$g 100 g$^{-1}$ f w were obtained. These results agree with research of other authors [29], who appliedd blue-LED supplementation to lettuce, and increased carotenoid content. As for the other cv., cv. Mantecosa registered values of 532 $\mu$g 100 g$^{-1}$ f w of $\beta$-carotene using $T_{0,}$ while cv. Romana registered 400 $\mu$g 100 g$^{-1}$ dry weight (d w) for the same light treatment. For $\beta$-carotene, in baby leaf lettuce other authors [30] obtained differences between light treatments using white, red and blue lighting, which was in good agreement with the results obtained in this work. Brazaityte et al. [11] reported that zeaxanthine is found generally in minor amounts in most vegetable species. In this work, zeaxanthine amounts were influenced by different light treatments [4]. Zeaxanthin was found in low values, and its contents were influenced by the different ligths in the following order $T_0 > T_1 > T_2$. Mantecosa registered values of 74 ($T_1$) and 79 ($T_2$) $\mu$g 100 g$^1$ f w, while cv. Angel showed 10 $\mu$g 100 g$^{-1}$ f w for $T_1$. $T_2$-treated Romana and Mantecosa cv. registered 1432 and 1373 $\mu$g 100 g$^1$ f w, and 919 and 836 ($T_0$) and 1192 and

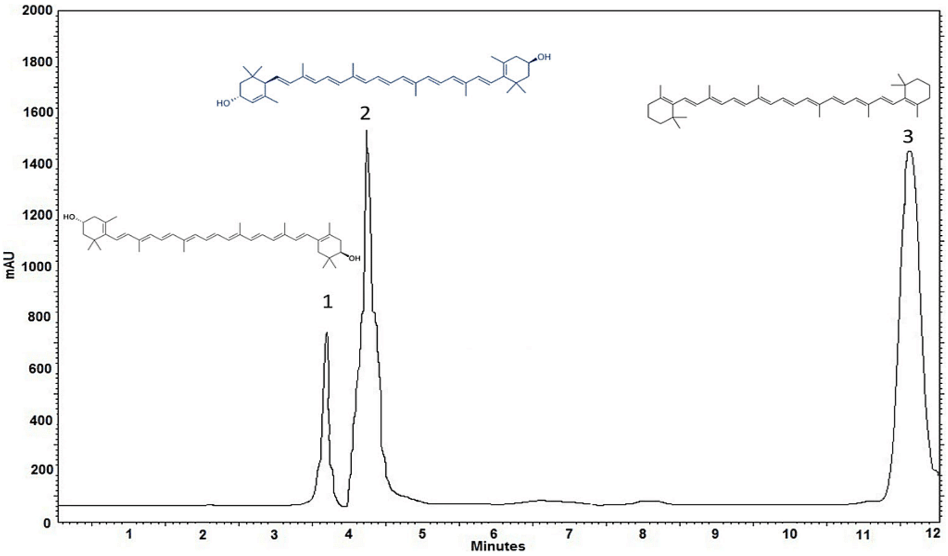


**Fig. 3** 476 nm-HPLC chromatogram of a lettuce cv. Mantecosa subjected to treatment $T_2$ sample. The standard components were eluded at different retention times with a sequential order: (1) zeaxanthin (3.7 min), (2) lutein (4.1 min) and (3) $\beta$ carotene (10.9 min)

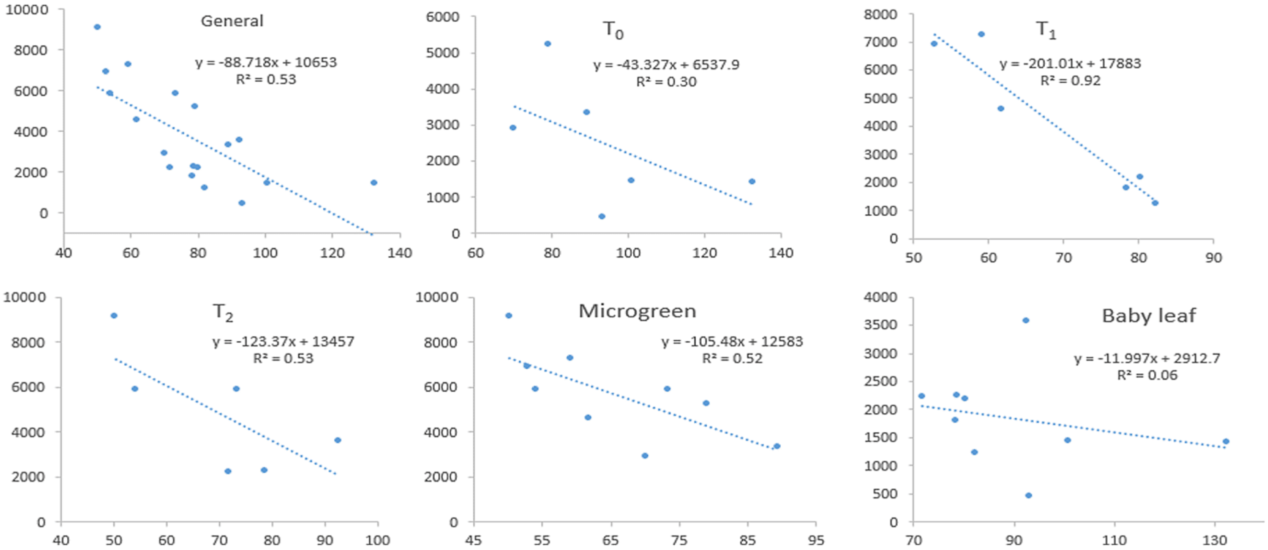


**Fig. 4** Lineal correlation between total carotenoid content in microgreens and baby leaf ($\mu$g 100 $g^{-1}$ fresh weight) and nitrate content of lettuce grown under different LED lamp. $T_0$ = L18 T8, $T_1$ = L18 NS1, $T_2$ = L18 AP67

1172 $\mu$g 100 $g^{-1}$ f w ($T_1$). Angel cv. showed lower amounts of lutein: 176, 630 and 811 for $T_0$, $T_1$ and $T_2$. These results agree with those of other authors [30] in baby leaf lettuce, who demonstrated the benefits of white, blue and red light on lutein in lettuce, when applying RB LED, RBW LEDS and white LED, respectively, using PFD of 210 $\mu$mol $m^{-2}$ $s^{-1}$. Moreover, in baby leaf the best values for lutein content were recorded in cv. Romana using $T_2$ light treatment (1432 $\mu$g 100 $g^{-1}$ f w) but this value was lower than at microgreen stage. This was also the case of cv. Mantecosa for the same light treatment, which in microgreens registered 4261 $\mu$g 100 $g^{-1}$ f w, vs. for baby leaf it was 1373 $\mu$g 100 $g^{-1}$ f w.

## Nutritional Implications of Nitrates and Carotenoids Content at Baby Leaf and Microgreen Stage

It was noted that nitrate and carotenoid content had an inverse relationship regarding developmental stage (Fig. 4), this was in T1 ($R^2 = 0.92$) in comparison to baby leaf stage where there was no correlation ($R^2 = 0.06$). In this study significant differences in carotenoid amounts were obtained due to light treatments: T0 registered the higher values of nitrate content for microgreen and baby leaf stages, and baby leaf stage reached the major amounts of nitrates for all light treatments and lettuce cv. Conversely, T2 induced higher carotenoid amounts, and at microgreens stage it was found the higher carotenoid amounts for all treatments and lettuce cv. At microgreens stage, nitrate values ranged from 50.2 to 89.4 mg 100 $g^{-1}$ f w, and carotenoids ranged from 17 to 5836 $\mu$g 100 $g^{-1}$ f w. Concerning baby leaf stage, nitrate ranged from 71.7 to 132.5 mg 100 $g^{-1}$ f w, and carotenoids ranged from 10 to 2103 $\mu$g 100 $g^{-1}$ f w. These values were specially favourables, given that according to USDA database, lettuce varieties have carotenoids amounts ranging between 277 and 299 $\mu$g 100 $g^{-1}$ f w. Carotenoid amounts in plant tissues were higher at the early stage of plant development (microgreens), which is in good agreement with findings of Chensom et al. [31], who reported that cilantro had a higher concentration of lutein/zeaxanthin and $\beta$-carotene at microgreens stage than that in mature ones.

Provitamin A carotenoids, *i.e.*, $\alpha$-carotene, $\beta$-carotene, and $\beta$-cryptoxanthin, can be metabolized in the body to retinol (vitamin A) [32, 33]. Research form showed a conversion by weight of 27:1 for $\beta$-carotene:vitamin A [34]. In contrast, no vitamin A activity can be derived from lutein, zeaxanthin, and lycopene. Considering all treatments and development stages, $\beta$-carotene was the main carotenoid in all samples. The higher $\beta$-carotene content was registered in T2-treated Romana cv. at microgreen stage, registering values of 5836 $\mu$g 100 $g^{-1}$ f w of $\beta$-carotene. The same treatment for cv. Angel microgreens registered values of 4500 $\mu$g 100 $g^{-1}$ f w $\beta$-carotene.

The equivalence between $\beta$-carotene and provitamin A is 1 IU of provitamin A equals to 0.6 $\mu$g $\beta$-carotene, and the recommended daily allowance (RDA) for vitamin A is 900 and 700 $\mu$g *per* day for men and women, respectively. Given our results, 42.80 and 33.2 g f w T2-treated Mantecosa cv. baby leaf and 15.42 and 11.99 g f w cv. Romana microgreens are enough to fulfil the recommeden daily allowance of provitamin A for men. This way, 100 g of T2-treated microgreen lettuce cv. Romana provides the 648.44 and 833.71 % of the total daily intake required of vitamin A for men and women, and 100 g of

T2-treated baby leaf Mantecosa cv. provides the 233.66 and 300.43 % of the total intake needed of vitamin A per day for adult men and women. Values of $\beta$-carotene for baby leaf lettuce obtained here are higher than values of fully-developed lettuce summarized in USDA database, which shows for lettuce cv. Romana, Mantecosa, Leaf and Crisphead 1560, 582, 114 and 198 Vitamin A ($\mu$g 100 g$^{-1}$), respectively.

In turn, LED-treated lettuce at microgreens-stage showed higher amounts of provitamin A than amounts detected in LED-treated baby leaf lettuce. Furthermore, LED-treated lettuce at microgreens stage registered higher amounts of carotenoids than fully-developed lettuce detailed in the USDA database.

## Conclusions

Vegetative growth of both microgreens and baby leaf lettuce stages were improved by red and blue light spectrum ($T_2$ and $T_1$), which induced higher fresh weight and height of all lettuce cv. From the point of view of health, lettuce consumption at microgreen stage is preferable compared to lettuce seedlings having more than 7–8 true leaves, due to that the nitrate in baby leaf approximately doubles content of nitrate at microgreen stage. LED light spectra $T_1$ and $T_2$ had a positive effect on growth and nutritional composition for all lettuce cv. and stages analyzed, especially continuous light-emitting diodes with red wavelength ($T_2$). Light type had different responses on productivity and nitrate contents, and was different depending on the lettuce cv. For instance, romana cv. reduced nitrates concentration using $T_1$ and $T_2$ with respect to the white light control ($T_0$), and $T_2$-treated lettuce cv. Romana registered the highest amounts of carotenoids, followed by cv. Mantecosa and Angel. $\beta$-carotene was the main carotenoid found in all cv. and stages, followed by lutein and zeaxanthin. For all lettuce cv., $T_2$-treated microgreens registered a significative higher content of carotenoids than baby leaf lettuce cv. Overall, daily vitamin A needs can be fulfilled by means of microgreens consumption: 100 g of $T_2$-treated microgreen lettuce cv. Romana provides the 648.44 and 833.71 % of the total daily intake required of vitamin A for men and women.

There was an inverse relationship between nitrate and carotenoid contents. At microgreens stage, nitrate amounts were lower than that found at baby leaf stage, while carotenoid amounts followed the opposite tendency. Hence, more research is required to found the ideal developmental stage of lettuce for higher carotenoids intake at lower nitrate amounts.

## Declarations

**Conflict of Interest** The authors declare that they have no conflict of interest.

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# 5. Algorithm implementation in Matlab for root measurement

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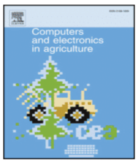

# Algorithm implementation in MATLAB for root measurement

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ABSTRACT

Modern agriculture has a growing level of technification, through the incorporation of new technologies of great utility in other disciplines different from the agricultural sector. The use of remote sensing systems to monitor the physiological state of crops by observing the volume and appearance of the roots is another example of its application. These systems are based on the detection and recording of spectral variations of crops both in the range of visible radiation and within the electromagnetic spectrum in the infrared range. Thermography is a visual tool that has found applications in agriculture. The monitoring of water status through the observation of crop roots is essential to optimize the use of water in agriculture, as well as its development and final production. The efficiency of root growth in basil crops has been analysed in a chamber with controlled humidity and temperature. The aim of this work was to demonstrate a better efficiency of root analysis with a first non-invasive and environmentally friendly method, visually, using thermal imaging and digital processing in the evaluation of radical growth versus two other conventional methods: traditional standard analysis and invasive analysis with digital image treatment. The results also showed advantages in the use of thermographic images compared to the use of RGB images for roots smaller than 1 mm.

## 1. Introduction

Modern agriculture must increase its production to feed the ever-growing population and is also undergoing continuous change to adapt to sustainable production. In order to do this, it has an increasing level of technification, through the incorporation of new technologies of great use in other disciplines different from the agricultural sector. For centuries, the existence of plant diseases has been one of the main limitations of agricultural production throughout the world. The search for control strategies that minimize production losses due to the pathogen, but at the same time guarantee agricultural sustainability, is now essential (Fang and Ramasamy, 2015).

The roots could access unevenly distributed chemical physical conditions, depending on the fertigation method, which can present considerable water savings, salts and substrate that make it more respectful of environmental sustainability (García-Caparrós et al., 2018; Sonneveld et al., 2011). If fertigation is combined with monitoring by sensors of humidity, temperature, electrical conductivity, pH, nitrates and potassium, this facilitates the choice of the distribution system for the application of nutrients in the substrate for better root development. Environmental monitoring has many applications with the aim of improving the sustainability of natural resources (Novas et al., 2017).

Traditional methods of root measurement are slow, costly and destructive and ineffective in presymptomatic identification of systemic pathogens (Fang and Ramasamy, 2015). New image processing technologies enable rapid and non-invasive measurement of root status, making it a powerful tool in detecting biotic and abiotic stress in numerous crops (Ishimwe et al., 2014). Currently, crop productivity measurement technologies mainly include direct measurement methods of field yield and methods of remote sensing and image processing (Chen et al., 2012). The imaging system has sufficient potential to provide more information compared to the system based on traditional sensors or measurement methods (Altmann et al., 2018).

With the use of thermography, a more in-depth investigation of water and nutrient availability can be conducted, resulting in greater growth of root length and volume (Urrestarazu et al., 2017).

In meeting long-term sustainability and environmental objectives, Vidyarthi and company research studies the use of infrared radiation as

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an alternative to traditional methods of studying water conservation and salinity management and quality (Vidyarthi et al., 2019). The thermography also has an increasing use in the field of food industry, work done by researchers such as (Chen et al., 2012), demonstrate the increasing accessibility of imaging technology, developing studies on systems for monitoring bee activity through the use of thermography. The main advantage of infrared thermal imaging is that it is non-invasive, contactless, without using the destructive nature of the technique to determine the temperature distribution of any object or process of interest in a short period of time (Vidyarthi et al., 2019).

The use of software to scan images and process them by algorithms developed in MATLAB is a very useful solution in this field of research. The use of algorithms for the analysis of images facilitates the work to obtain meaningful conclusions. This results in a combined use of different image analysis techniques which will help to extract the maximum information on the different physiological and biochemical parameters of plants such as photosynthesis, chlorophyll, or water use efficiency (McAusland et al., 2013). Another fundamental aspect to be considered for the optimization of the use of image processing will be the improvement of data analysis and processing systems that facilitate the making of conclusions and decisions by the farmer (Altmann et al., 2018).

In this work three different methods of analysing the quality of the roots has been tested to obtain the physiological and quality status of the plants. The main objective of this article is to demonstrate that through an indirect method of non-invasive image processing and use of algorithms in MATLAB, similar area and volume results are achieved as with traditional methods. Through the use of indirect and non-destructive analytical methods for the plant, it is possible to know the physiological state of the root. For this, the results obtained from using the three methods were compared between them. There is the traditional direct root measurement method and two indirect methods: one rendered the plant unusable and the other conservative by thermography. This last proposed method is considered a non-invasive indirect method, which avoids the destruction of the natural environment to be analysed, and therefore, respecting nature. Proving the use of thermographic images in conjunction with digital image processing and programming techniques is a useful tool for automating the analysis of sustainable crop production.

## 2. Material and methods

The tests were carried out in the laboratory of Climate and Soilless Culture of the CITE V of the University of Almeria (Almeria, Spain), in the controlled environment chamber (10 m × 2.5 m).

Experiments were carried out on basil (*Ocimum basilicum* L.) and plants were fertigated with a standard nutrient solution based on that described by (Sonneveld et al., 2011), with an E.C. of 2.2 $\mathrm{dSm}^{-1}$ and a pH of 5.8. Irrigation was applied every 2 days, once the limit of 10% of the water readily available for the substrate was reached.

For the calculation of the vegetative parameters of the roots, a first standard method was used to measure the volume, length, diameter, dry and fresh weight of the roots with a conventional millimeter pitcher, a caliper (Calibre Digital Tacklife® DC02 150 mm) and a conventional tape measure.

The two methods of measuring the root area were done by capturing the images: thermography and RGB camera photographs.

Each imaging method used an area measurement system with two different softwares: the ImageJ program was used for thermography (Schneider et al., 2012) and an application developed under the MATLAB environment was used for the RGB photographs (Mathworks, 2018).

The thermographic images were recorded with a compact infrared camera, the Fluke® Ti32 thermal imaging scanner (Janesville, WI, USA), with an infrared spectral range of 7.3–14 μm, a temperature range of −20 to +600 °C and an accuracy of ± 2%. The camera used is compatible with the SmartView 4 software package 3™ (Fluke Thermography, Plymouth, MN, USA).

The RGB camera selected is the Huawei P10 Lite camera with a resolution of 11.8 Mpx and a pixel size of 1,250 μm. An algorithm was programmed for processing using MATLAB 2016 (Mathworks, 2018) software.

### 2.1. Statistical analysis

Vegetative and image analysis data were subjected to a variance analysis and their averages were compared to a Tukey test at $P \leq 0.05$ using Statgraphics Centurion® (Warrenton, 2018).

## 3. Experiments design

The emissivity range, $\mathscr{E}$, was defined as 0.98 according to literature (Rahkonen and Jokela, 2003). The crops were separated inside a chamber with controlled parameters of humidity (50% Hr), temperature (21 °C) and programming of 16 h of daily light.

The experimental design was in complete random blocks with 15 repetitions with 2 plants per repetition for a single treatment.

For taking the photographs, a controlled lighting of 350 Lux was used, an anti-reflective background, a drainage system to apply fluids at different temperatures for taking the thermal photographs and a measurement reference was added to the image for later analysis.

Among the additional considerations was determined a distance for taking photographs in order to represent the largest amount of visual information of the crop. The following photographs were taken:

A. Front and back RGB photography.
B. Far front and far back thermal photography.
C. Front close and back close photography.

Considering a distance for A and B of 55 cm (cm), the frontal photograph, the back photograph, and a distant photograph were obtained (Fig. 1), in all the photographs it was observed in a contrasting way, the measurement reference, which allowed us to obtain a pixel-measurement ratio for the size analysis in MATLAB.

For the photograph captured at distance C a distance of 35 cm was defined, in addition water is applied to them at two different temperatures, of 35 °C and −18 °C. The result of changing the temperature to the substrate is presented as an advantage for analysis because there is a greater thermal difference between the substrate and the root with a value of ± 1.8 °C.

### 3.1. Measurement of roots by traditional method

The volume of the roots, the diameter, length, fresh and dry weight were measured. The roots were washed and classified according to the distribution of their root volume, measured by water displacement according to the traditional method currently used by (García-Caparrós et al., 2018).

Plants were randomly selected to measure morphological variables: stem length, diameter, fresh and dry weight, and root volume. The plants were then dried for 48 h at 78 °C to determine the biomass of each of its components.

Variance analysis was used to determine differences in morphological variables between root volume categories and the rest of parameters.

### 3.2. Area measurement with Image J software

ImageJ® was used to calculate the root area using thermographs (Schneider et al., 2012). It is an open source digital image processing program, free access and programmed in Java by the National Institutes of Health of the United States (https://imagej.nih.gov/ij/). It allows

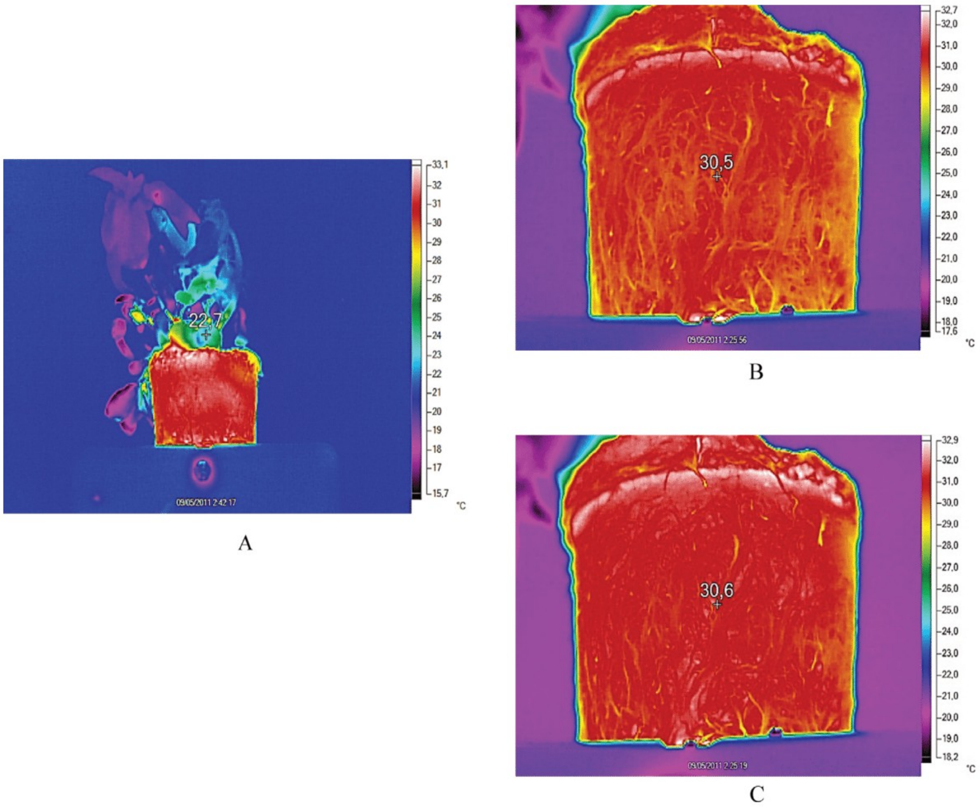

**Fig. 1.** Thermography images: A. (far front), B. (close front) and C. (close back) of a pot under their temperature gradient.

personalized scanning, analysis and processing using the editor included in ImageJ, as can be seen in Fig. 2. It allows the incorporation of plug-ins and applications made by other users which makes it very versatile.

To obtain the root area, as shown in Fig. 2, proceeded as follows:

1 *Improve the image (step 1 of* Fig. 2*).* The image is used to quantify the signal, having to keep the original image, since any retouching produces changes in the pixel values.

The most common retouching has been used as a contrast change. The Auto option gave an acceptable result. The graph shown is the “histogram“ and represents in X the possible values of the pixels (from 0 to 255) and in Y the number of pixels that have a certain value. The histogram gave an idea of how illuminated an image is.

*2. Enter the measurement data manually:* Image J automatically incorporated the image data (metadata). This data contained the calibration of the image and then a measurement bar in the menu was inserted. As the image didn't have the measurement data, they have been entered manually. To do this it was required to take an image of a micrometre or a tape measure in the same conditions as our image was taken and counted how many pixels represent a distance in centimetres.

In order to know how many pixels, represents a known distance, first, the image in which the taped measure appears was opened. The Image J bar used the straight-line tool and drew a line between two scale marks. The pixel and distance data (200 pixels represent 100 µm) were entered by using the option Set scale. In Unit of length it was put cm.

*3. Quantify the signal and adjust the brightness and contrast values.* Since the screen didn't have the measurement data, it was entered it manually. To do this it was required to take an image of a target micrometre or tape measure in the same conditions as our image was taken and count how many pixels represent a distance in microns.

For greater convenience when working, from the menu Analyse > Tools > Scale Bar, ImageJ allowed you to choose the size of the bar, as well as its position, colour, and font among others.

*4. Open the target micrometre or tape measure image and select the area.* Then it could measure on the image or inserted the measuring bar in *Scale bar*. This was followed by the analysis of the selected area using the red contrast, *-analyse, measure*.

The tool *Point tool* was selected in order to click on the structures (roots) to count them. On the emerging window the mark type was chosen (dot, circle, cross, etc.), using colour and size, for this case the cross to select the whole area was selected.

*5. Analysis of the selected area with red contrast.* The program measured the area and provided us with a table of values with the area in the unit corresponding to the scale it was entered in step 1.

*Obtaining data.* Because of they were made numerous measurements, in the same Results window the option Summarize calculated the average (Mean), standard deviation (SD), minimum (Min) and maximum (Max) values. Subsequently the program provided a window of results for the measurements, these data could be saved (it was a table of any program for calculation and data processing), from File menu of the same window.

### *3.3. Measurement of root area under the MATLAB environment*

For this application a MATLAB software has been developed in order to process the images taken with the thermographic camera both in thermographic and RGB format, as shown in Fig. 3. The processing was done in 5 steps obtaining finally an image to measure the area of the photographed roots.

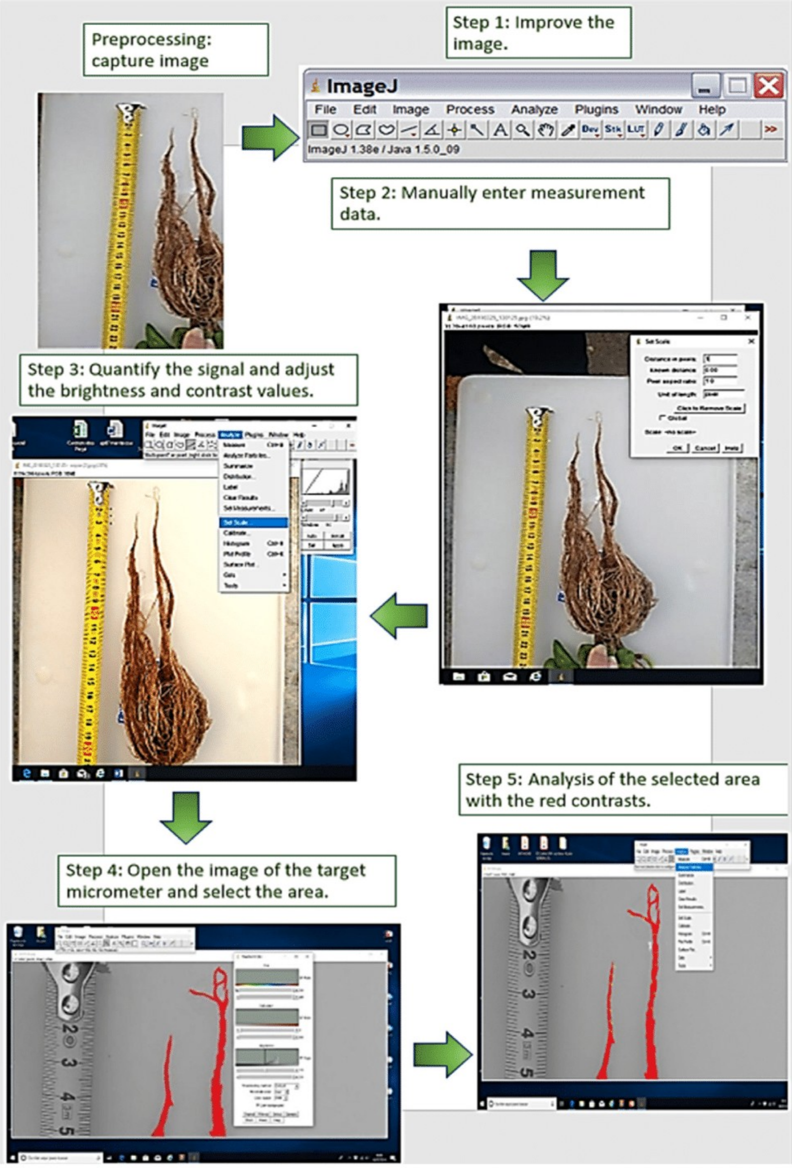

**Fig. 2.** Root analysis by Image J.

1. *Change from RGB to grayscale.* Using the rgb2gray function in MATLAB converted the TrueColor RGB image into the grayscale I image. The rgb2gray function converted RGB images to grayscale eliminating hue and saturation information while retaining luminance.

Syntax

I= rgb2gray(filename. jpg)

newmap = rgb2gray(I)

2 *Filter TopHat.* Top-hat transformations have been used to process images, in background equalization and image enhancement.

Function *f*: E-R being a grayscale image, assigning points from a Euclidean space or a discrete E grid (such as R2 or Z2) to the real line. You had let b(x) be a grayscale structuring element.

Then, the white transformation of the top hat of *f* was given by:

T_w (f) = f– f ˚ b

Where the number indicated the opening operation.

The black transformation of the top hat of *f* has been given by:

T_b (f) = f■b-f

Where ■ was the closing operation.

3 *Contrast Adjustment.* The morphological filtering of the upper hat has been performed in the gray scale or binary image I, returning the filtered image.

Syntax

J= imtophat(I, SE)

J= imtophat(I, nhood)

4. *Binarize.* BW - imbinarize(I) created a binary image from the 2D

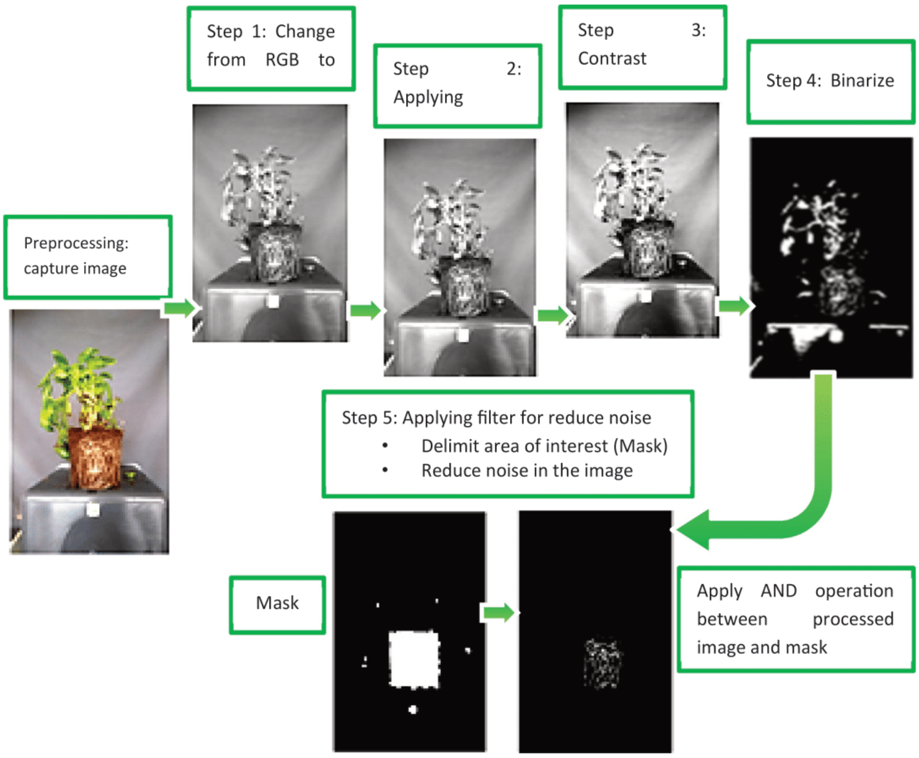

**Fig. 3.** Root analysis by MATLAB.

or 3D I grayscale image by replacing all values above a globally determined threshold by 1 s and setting all other values to 0 s. By default, imbinariz uses Otsu's method, which chose the threshold value to minimize the intraclass variance of the black and white threshold pixels.

Syntax

BW = imbinarize(I)

Filters were required to reduce noise:

I. Erosion of a binary image f by structuring elements, s (denoted s) produced a new binary image g-f s with which are indicated in all locations (x,y) of the origin of a structuring element where that structuring element fits the input image f, i.e.: g(x,y) a 1 is s is set to f and 0 otherwise, repeating for all pixel coordinates (x,y).
Erosion with small square structuring elements (e.g. 2 × 2 to 5 × 5) reduced an image by removing a layer of pixels from the inner and outer boundaries of regions. Holes and voids between different regions became larger, and small details were eliminated.
II. The dilation of an image f by a structuring elements (denoted f s) has produced a new binary image g-f s which are indicated at all locations (x,y) of the origin of a structuring element where that structuring element s hits the input image f, i.e. g(x,y) 1 if s hits f and 0 otherwise, repeating for all pixel coordinates (x,y). Dilation has the opposite effect to erosion: it adds a layer of pixels to the internal and external boundaries of the regions.

The holes enclosed by a single region and the gaps between different regions became smaller, and small intrusions were filled into the boundaries of a region.

*5. AND operation.* Applying the AND operator (or other logical operators) to two images to detect differences or similarities between them was more appropriate if they are binary or can be converted to binary format by threshold.

The pixels that share both images have been preserved, the other pixels have been discarded. This technique served as a filter to calculate the area of interest within an image (count the resulting pixels).

Obtaining the real area is based on the number of pixels resulting from processing and applying a measurement factor according to an object as a reference in our unprocessed images. In our case, a coin was used as reference, of which it was known its real size and therefore its equivalent in pixel. The process was as follows:

Found the scale factor:

$$f = d_{image}/d_{benchmark} \tag{1}$$

For the final calculations it was used as example the diameter of a coin in mm and divide it by the scale factor previously estimated.

$$diameter_{final} = diameter_{initial}(mm)/f \tag{2}$$

In this way, the reference diameter of the image was known (final diameter) and starting from it is applied as a scale factor of the image to each final pixel (obtained after applying the 5 step).

## 4. Results and discussion

### 4.1. Results

The fresh matter weight of the roots was an average of 65 g plant$^{-1}$, proportionally the dry matter weight was maintained at an average of 7 g plant$^{-1}$, which demonstrated a relationship with the volume presented for each basil plant tested (Fig. 4). The volume of the roots was directly related to the vigour of the roots of the crop, these being the main axis of the development of the plants, besides being indicators of the physiological state or of the existence of hydric stress or appearance of diseases of the crops. As shown in Fig. 4, the volume did not exceed 100 ml plant$^{-1}$ with the exception of two of the units tested (M2), at

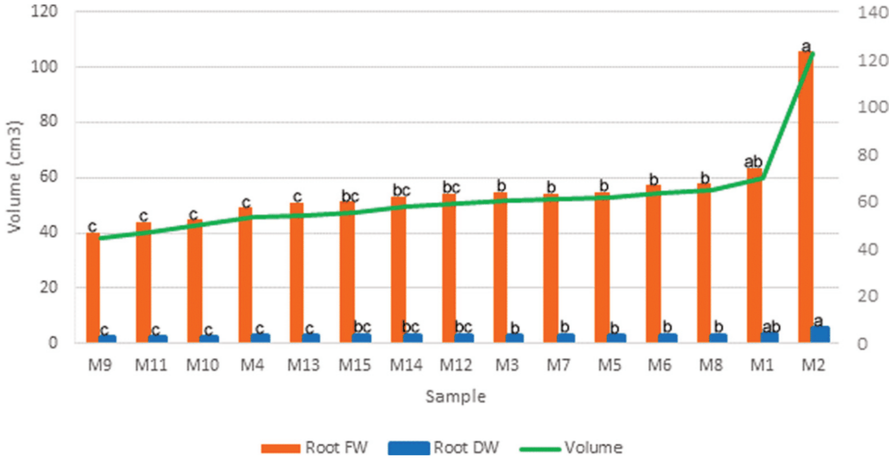

**Fig. 4.** Comparative graph of the fresh and dry weight of the root with the volume of each plant. Different letters indicate significant differences between vegetative parameters of fresh and dry weight in a Tukey Test at $P < 0.05$.

equal conditions of development and cut, which demonstrated a fixed proportion of growing conditions. In order to have a better view depending on the increase in volume, the samples have been ordered from lowest to highest, represented by the nomenclature M1, M2, etc. Both the dry weight and the fresh weight were similar in the plants tested. It was correlated very significantly with the volume values obtained, as can be seen in Fig. 4. The data was presented according to the number of the sample analysed or repetition, this being composed of two plants per sample, and expressing the unit of this repetition or sample as the average of the values of two independent pots and chosen at random within the same experiment. Thanks to the measurement of roots which was more significant than the observation of other parts of the plant in principle, this analysis could be complemented with the measurement of leaf area and vegetative parameters of the stem, which would remain a sustainable method with the environment and crops and non-destructive.

The unit of time for the entire trial was an average of 25 days. Crop yields, as shown in Table 1, were normal for this plant variety according to the literature studied (Ortega and IvÃ, 2012). In all the cases analysed, the root length correlated with the volume presented, not necessarily maintaining this relationship with the fresh weight parameters for stem and leaf. However, there was a notable relationship between root length, fresh weight, dry weight and diameter, although there were no differences of more than 3% between the diameter of the different units of the crop. The dry matter contents of the offspring per plant reflected the differences between the cultivation units composed of 2 plants per repetition, resulting in most cases, significant.

Fig. 5 shows the data obtained using the two computing tools (Image J and MATLAB Programming) and their comparison with the volume calculated by the traditional procedure. If the results of the areas obtained are compared, there is a slight difference between the results obtained by the Image J analysis program and the use of the algorithm in the MATLAB environment. In all cases, the results of ImageJ were slightly higher (4.5% on average) than those of the algorithm developed in MATLAB, this is due to the subtraction of the superposition of images to which MATLAB was subjected, but, despite this, the accuracy of the data analysed by MATLAB, was remarkably significant and better than the precision conferred by the analysis with Image J, because for the human eye, it is more difficult to separate the root of the substrate in the image, unlike for MATLAB. To validate the results, the results obtained by image processing were compared with those obtained with the traditional direct method. The average values of volume were very similar between the culture units evaluated, existing relation of proportionality between them and the area of the plant measured in both techniques. This showed an equivalence and therefore, indicated us that it is possible to use indirect analytical methods to

**Table 1**
Results of measurements on a basil crop 25 days after cultivation. Different letters indicate significant differences between vegetative parameters in a Tukey Test at P less than 0.05.

| Measurements | | | Fresh weight | | | Dry weight | | |
|---|---|---|---|---|---|---|---|---|
| Samples | Length (cm) | Root diameter(mm) | Stem (g) | Leaf (g) | Total (g plant$^{-1}$) | Stem (g) | Leaf (g) | Total (g plant$^{-1}$) |
| M1 | $56,90_{ab}$ | $1,00_{a}$ | $22,82_{a}$ | $34,34_{b}$ | $120,30_{b}$ | $3,00_{b}$ | $3,90\ _{cd}$ | $10,29_{b}$ |
| M2 | $70,50_{a}$ | $1,03_{a}$ | $10,94_{d}$ | $22,35_{d}$ | $138,92_{a}$ | $1,60_{d}$ | $2,20_{d}$ | $9,60_{c}$ |
| M3 | $50,70_{b}$ | $1,00_{a}$ | $18,03_{b}$ | $30,30_{bc}$ | $103,00_{c}$ | $2,40_{c}$ | $4,00_{c}$ | $9,21_{c}$ |
| M4 | $43,00\ _{cd}$ | $0,96_{b}$ | $21,28_{a}$ | $34,09_{b}$ | $104,73_{c}$ | $3,58_{b}$ | $5,00_{b}$ | $11,18_{b}$ |
| M5 | $52,20_{ab}$ | $1,00_{a}$ | $19,74_{a}$ | $37,83_{a}$ | $112,50_{bc}$ | $3,10_{b}$ | $5,20_{ab}$ | $11,15_{b}$ |
| M6 | $55,00_{ab}$ | $1,00_{a}$ | $12,80_{c}$ | $22,23_{d}$ | $92,26_{d}$ | $1,80\ _{cd}$ | $2,45_{d}$ | $7,24\ _{cd}$ |
| M7 | $51,20_{ab}$ | $1,06_{a}$ | $12,49_{c}$ | $22,31_{d}$ | $89,12_{d}$ | $1,88\ _{cd}$ | $2,30_{d}$ | $6,95_{d}$ |
| M8 | $56,00_{ab}$ | $1,00_{a}$ | $16,56_{b}$ | $26,72_{c}$ | $101,47\ _{cd}$ | $2,20_{c}$ | $3,30\ _{cd}$ | $8,51\ _{cd}$ |
| M9 | $35,00_{d}$ | $0,94_{b}$ | $16,48_{b}$ | $30,12_{bc}$ | $86,70_{d}$ | $2,50_{bc}$ | $4,80_{bc}$ | $9,36_{c}$ |
| M10 | $40,00\ c_{d}$ | $0,95_{b}$ | $23,56_{a}$ | $40,55_{a}$ | $109,25\ _{cd}$ | $4,30_{a}$ | $6,70_{a}$ | $13,37_{a}$ |
| M11 | $38,90_{d}$ | $0,90_{b}$ | $15,78_{bc}$ | $32,74_{bc}$ | $92,62_{d}$ | $2,20_{c}$ | $3,50\ _{cd}$ | $8,00\ c_{d}$ |
| M12 | $49,13_{ab}$ | $0,97_{ab}$ | $18,50_{b}$ | $26,12_{c}$ | $98,80_{d}$ | $3,00_{b}$ | $3,05\ _{cd}$ | $8,85\ _{cd}$ |
| M13 | $43,30_{c}$ | $0,94_{b}$ | $23,93_{a}$ | $34,29_{b}$ | $109,03\ _{cd}$ | $4,00_{a}$ | $5,50_{ab}$ | $12,13_{a}$ |
| M14 | $47,00b_{c}$ | $0,96_{b}$ | $13,23_{bc}$ | $28,18_{c}$ | $94,61_{d}$ | $2,40_{c}$ | $3,45\ _{cd}$ | $8,64_{c}$ |
| M15 | $46,10_{bc}$ | $0,93_{b}$ | $15,34_{b}$ | $38,23_{a}$ | $105,07\ _{cd}$ | $2,10_{c}$ | $4,20_{bc}$ | $8,98_{c}$ |

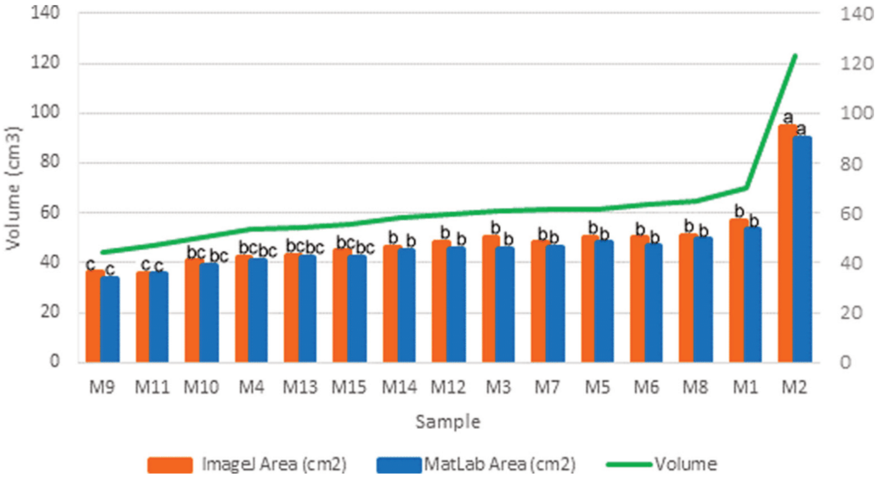

**Fig. 5.** Comparative graph of the analysis by Image J and analysis by MATLAB with the volume of each plant. Different letters indicate significant differences between ImageJ area and MATLAB area in a Tukey Test at $P \leq 0.05$.

check the good physiological state and quality of the plants of the variety studied.

Fig. 6 shows the equivalence value between the volume measured by the traditional method and the two methods of image processing, with the samples sorted from lowest to highest measured volume. For the calculation with Image J the average equivalence was 1.2495 cm and for MATLAB it was 1.3094 cm. Making an error in all cases less than 4.3% (see Fig. 7, M11 for Image J), less than 2 ml difference volume. The volume could be calculated indirectly by applying the linear deviation equation for its simplicity:

$$y = Ax \quad (3)$$

For $x$ represented by area, and the volume of the sample and A the conversion coefficient. To obtain the coefficient, the average of the division of the volume (calculated by traditional method) with respect to the area calculated with the image processing tools was calculated. Fig. 7 presented the averages with their coefficients, obtaining the following values for each coefficient:

$$A_{ImageJ} = 1.2495$$

$$A_{MatLab} = 1.3094$$

Fig. 8 shows the cumulative conversion error. A Pearson correlation coefficient (ρ) of 0.994416234 for Image J and 0.997472186 for MATLAB was obtained, showing non-significant differences between the value obtained from the volume by the traditional method and that calculated indirectly applying Eq. (3) and its coefficient as the case may be. Other types of equations could be applied, but they complicate the calculation of the coefficients and little difference resolution is gained. The standard deviation of cumulative errors (area calculation error and volume conversion error) is 2.85% for Image J and 1.88% for MATLAB, which showed that MATLAB makes fewer cumulative errors than Image J.

It was intended to relate the error to the volume value, the higher the volume value, the greater the error in the case of MATLAB, although this was not always the case for Image J. As can be seen in graph 8, MATLAB made a smaller error than Image J when calculating the volume. The error could be reduced proportionally if the number of plants or culture units tested had been increased. In the values it can be observed how there is an error by excess, resulting positive or negative depending on the plant evaluated, or by default.

### *4.2. Discussion*

The accuracy of the obtained values improved the values obtained by (Lukac and Godbold, 2001), presenting a key disadvantage through the use of rhizotrons for the estimation of these values, which included continuous and substantial disturbance of the roots, which are of vital importance for the development of the crop.

When having an exhaustive analysis of the crops, it is possible to complement this non-invasive method with an analysis of leaf area and measurement of stem diameter, stem length, number of leaves, among other non-invasive parameters as well, being equally respectful with the environment and sustainable, when controlling the sample space of the

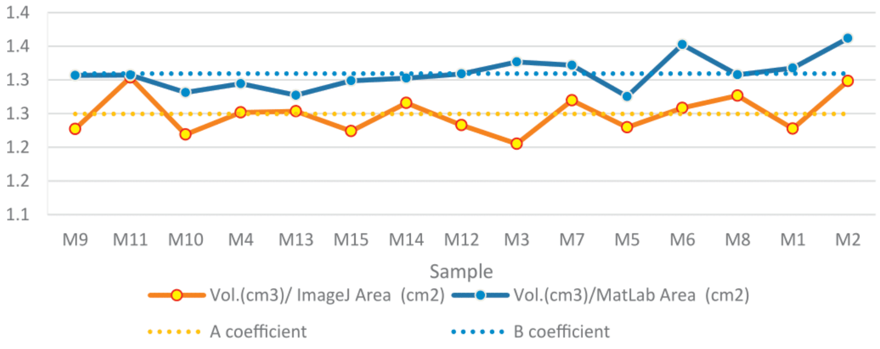

**Fig. 6.** Comparative graph of the equivalence in cm between Vol.(cm$^3$)/Area (cm$^2$).

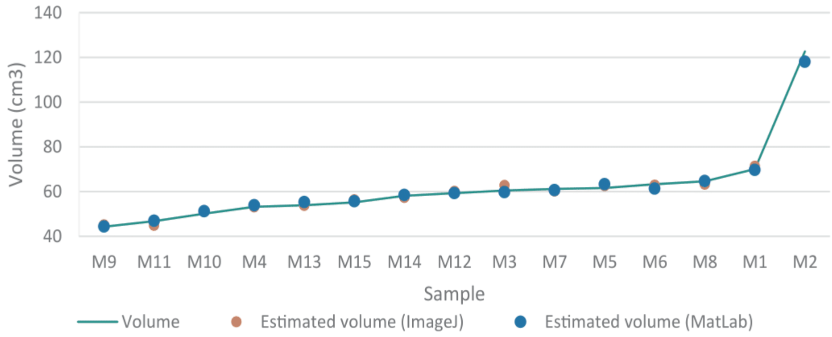

**Fig. 7.** Distribution of estimated volume value.

crops, bearing in mind that the values vary according to the species and variety of crop tested.

Authors such as (Callejas-Rodríguez et al., 2012), explain the close relationship between the volume and vigor of the root and the development of the plant, especially in roots with less than 1 mm in diameter, despite the fact that a great deal of time and work is needed to obtain these data. When predicting water stress or disease occurrence, root observation has important advantages.

The unit of time for the entire trial was, on average, 25 days. The obtained crop yields were normal for this variety according to the studied bibliography (Ortega and IvÃ, 2012).

The dry matter contents of the offspring for each plant are summarized in Table 1 and reflect the differences between the crop units -composed of 2 plants per repetition - were not significant.

As the root volume increased, so did the morphological parameters evaluated. The improvement of a root system of greater volume and is very useful to capture more nutrients from the soil, which is essential for the survival of the crop, in conditions of water deficit. The volume of root systems is becoming increasingly important in agronomy and they are concentrating a great deal of research because of their enormous potential to promote sustainable development and crop production (Urrestarazu et al., 2017).

The stem to root ratio was affected by root volume, which means that the plants maintained a balance in their water absorption and transpiration ratio despite having different root systems in size.

Both fresh and dry weight were similar in the plants tested. It correlates very significantly with the volume values obtained, as can be seen in Fig. 4. Also (Urrestarazu et al., 2017) obtains similar results in the analysis by means of thermography of fresh and dry weight in roots.

The total dry matter production was the result of the efficiency of the crop foliage in the interception and use of the available solar radiation during the growth cycle, however, it is not directly related to the root length, but is influenced by other external factors, this matches with the experiments carried out by Jerez Mompies and Martín (2012) in potato cultivation.

The most productive plants were associated with a greater quantity of roots, with a greater volume of soil occupied. In some cases, there was no significant relationship between root volume and fresh weight of leaves and stems, this coincides with research conducted by (Domínguez et al., 2019), who states that individuals plants with higher chlorophyll content in their leaves are able to have higher rates of photosynthesis, greater carbon gain and as a final result a higher growth of the offspring, both leaves and stems, and there is also an ontogenetic effect that is superimposed on the general pattern.

The measurements analysed with Image J have higher values because the program allows you to select the pixels to count, which made the measurement of very small capillaries more accurate. The main drawback is that an enormous amount of time must be invested by an expert user in this analysis program. This does not happen with MATLAB, where you spend a lot of hours programming the analysis algorithm and few times analysing it. In practice, for the 5% difference between the two programs, the performance in number of hours of dedication is much better in MATLAB.

MATLAB produces a smaller error when calculating volume conversion when measuring averages than Image J, this is due to the fact that photographs of the roots are intermingled with the substrate, which makes it difficult for the human eye to discriminate between root and soil.

Authors such as (Schneider et al., 2012) agree on these results, endorsing the use of Image J for the measurement of diameters and areas of plant elements, especially the roots, because it facilitates the measurement function by means of a freehand line or segmented lines. The application of required threshold values with the threshold function of the Image J software was very useful to reach the accuracy in the measurement of the values when quantifying pixels to obtain an area in cm$^2$, investigations carried out by (González, 2018) for the

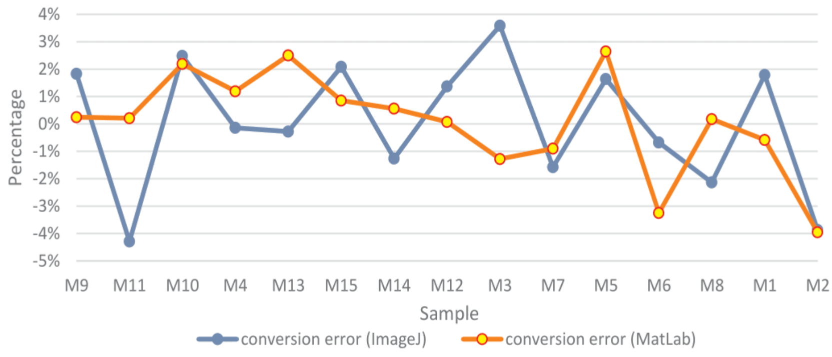

**Fig. 8.** Comparative graph of the errors made from conversion to volume.

measurement of pores of images taken by electron microscopy, coincide with the accuracy of the values obtained.

The accuracy of the root area values measured through Image J is higher than values of MATLAB, also because the program has admitted in the analysis of this experiment all common image manipulations, including reading and writing image files, and operations on individual pixels, root image regions, through full images and volumes.

The data obtained from the root area has been shown to do post-processing algorithms, similar results were investigated by (Sanquin et al., 2019), proving the improvement in the performance of the measurement of the area by images through algorithms in MATLAB.

Root area was recorded with an error of less than 5% between the results obtained for the Image J and MATLAB analysis program, observing that there are no significant differences between the area obtained using MATLAB and the average area obtained.

## 5. Conclusions

Three methods have been compared, one non-invasive, one traditional and one by image analysis. The comparison showed that the non-invasive method (thermography and MATLAB image processing) is a useful tool for detecting roots of a size smaller than 1 mm, which is not possible with the other two methods.

Subsequently, a better efficiency of root analysis was found with a non-invasive and environmentally friendly method. Among the three methods studied, the use of thermography combined with processing in MATLAB is more effective. MATLAB produced a smaller error when calculating volume conversion of measured averages than Image J. Moreover, MATLAB made fewer cumulative errors than Image J, due to the standard deviation of cumulative errors was 2.85% for Image J and 1.88% for MATLAB.

The area of the plants showed positive correlation with the volume of the same ones and an indirect method of calculating the volume of the root has been obtained without necessity of destroying it, a suitable size and volume of root assures a better development and production of the culture. Through the use of control plants in pots, the application of the method could be extended in soil crops, in addition to hydroponics.

The results show the viability of being able to control the evaluation and monitoring of the physiological state of the plant. The volume of the root system is essential for the development and production of these, so the importance of their study, if it provides optimal moisture and nutrition, well-developed roots will send hormonal type signals that will enhance the development of shoots and fruits. The main limitations for the adequate development of the volume of the root are physical and will depend on the management of the soil and irrigation water. Therefore, monitoring and analysis are essential for proper crop management.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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# IV Conclusions

## Conclusiones

Se ha realizado un significativo beneficio del cultivo del arándano a través de la aplicación de Si en el fertirriego que pudiera ser extrapolable a otros cultivos hortícolas. Describiendo algunas importantes consideraciones en las rutas metabólicas implicadas.

Se ha determinado la mejor concentración de amonio en la solución nutritiva para un cultivo de pimiento en condiciones de cultivo sin suelo. Definiendo que es importante encontrar la dosis exacta para cada cultivo y condiciones específicas no solo del cultivar sino del medio de cultivo y su manejo agronómico.

Se ha mejorado y aplicado ciertos algoritmos en MATLAB para medición de raíces. Demostrándose que muchos medios matemáticos desarrollados en otras tecnologías pueden contribuir significativamente como herramientas útiles al estudio y contribución junto al fertirriego del crecimiento radical y por tanto de la producción total y de calidad.

La combinación de las técnicas de fertirrigación y la luminotecnia sostenible LED adecuada aumenta significativamente los beneficios de la composición fitoquímica y nitratos en la lechuga. Abriéndose la posibilidad de extrapolar esta conclusión a otras técnicas y cultivos hortícolas.

# Conclusions

A significant benefit has been realised for the blueberry crop through the application of Si in fertigation that could be extrapolated to other horticultural crops. Some important considerations in the metabolic pathways involved have been described.

The best ammonium concentration in the nutrient solution for a pepper crop under soilless conditions has been determined. Defining that it is important to find the exact dosage for each crop and conditions specific not only to the cultivar but also to the growing medium and its agronomic management.

Certain MATLAB algorithms for root measurement have been improved and applied. It has been demonstrated that many mathematical means developed in other technologies can contribute significantly as useful tools to the study and contribution together with fertigation of root growth and therefore of total production and quality.

The combination of fertigation techniques and appropriate sustainable LED lighting technology significantly increases the benefits of phytochemical and nitrate composition in lettuce. This conclusion can be extrapolated to other techniques and horticultural crops

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# VI Scientific contributions

Publications

1. Urrestarazu, M., Gallegos-Cedillo, V., Ferrón-Carrillo, F., Guil-Guerrero, J.L., Álvaro, J.E., Lao, M.T., 2019. Effects of the Salinity under Soilless Culture Systems on Gamma Linolenic Acid Levels in Borage Seed. PLoS ONE. 14(2), 1–9. https://doi.org/10.1101/454405. Q1 Journal.

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6. Aliniaeifard, S., Ghorbanzadeh, P., Esmaili, M., Azadegan, B., Ferrón-Carrillo, F., Lastochkina, O., Li, T., Seif, M., 2020. $CO_2$ enrichment and increasing light intensity till a threshold level, enhance growth and water use efficiency of lettuce plants in controlled environment. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 48. 2244–2262. https://doi.org/10.15835/nbha48411835. Q2 Journal.

7. Ferrón-Carrillo, F., Urrestarazu, M., 2021. Effect of Ammonium Nitrogen on Pepper Grown Under Soilless Culture. Scientia Horticulturae. Q2 Journal.

8. Esmaili, M., Mashal, M., Aliniaiefard, S., Urrestarazu, M., Ferrón-Carrillo, F., 2021. Impact of Silicon on Chemical Properties of Drainage Water from Lettuce Following

Determination of Proper Cultivar and Light Spectrum. Communications in Soil Science and Plant Analysis. https://doi.org/10.1080/00103624.2020.1869762. Q2 Journal.

9. Ferrón-Carrillo, F., Guil-Guerrero, J.L., González-Fernández, M.J., Lyashenko, D., Battafarano, F., Pagan Loeiro da Cunha, T., 2021. LED enhances plant performance and both carotenoids and nitrates profiles in lettuce. Q1 Journal.

Transference merits

1. Name of the contract: Agronomic evaluation of the use of silicon as a nutrient in a soilless cultivation system to improve the productivity and quality of blueberries. 2.
agronomic evaluation of the use of silicon as a nutrient in a soilless cultivation system to improve the productivity and quality of blueberries. Code: 4001237. Scope: International. Funding entity and programme: Rocio Group. TAL SA.
Principal Investigator: Urrestarazu Gavilán, Miguel. Start date: 02/01/2017-Finish date: 31/12/2018. Amount (EUROS): 22185.00. Relation to the project: Closely related: management of plants sensitive to salinity.

2. Name of the contract: Agronomic evaluation of the use of silicon as LABINSINERGIC in different horticultural various horticultural species
Code: 401273. Scope: European. Financing entity and programme: MACASA SA. Principal Researcher: Urrestarazu Gavilán, Miguel. Start date: 01/01/2017. End date: 30/07/2018. Amount (EUROS): 20.682,69. Relation to the project: Closely related: management of brackish water and the palliative effect of Si on water-deficient crops.

3. Name of the contract: Evaluation of ammonium toxicity in a pepper crop on coconut fibre.
on coconut fibre. Code: 401273. Scope: European. Financing entity and programme: MACASA SA. Principal Researcher: Urrestarazu Gavilán, Miguel. Start date: 01/01/2017. End date: 07/30/2018.

Amount (EUROS): 20,682.69. Relation to the project: Closely related: management of brackish water and the palliative effect of Si on water-deficient crops.

4. Name of the contract: Sustainable production of new borage species for the production of functional oils rich in gamma linolenic acid. of functional oils rich in gamma linolenic acid.
Code: 67528. Scope: European. Funding entity and programme: AGL2015- 67528-R. Principal Investigator: Urrestarazu Gavilán, Miguel. Start date: 01/01/2017.
Date end: 31/01/2018. Amount (EUROS): 20,000.09. Relationship to project: Closely related: Effect of Si on soilless crops.

Teaching

1. Study Abroad 2019. Internationalisation Programme. Advanced Horticulture in Almería.
Fertigation. 1-12 April 2019. 24 hours.
2. Study Abroad 2019. Internationalisation Programme. Advanced Horticulture in Almería.
Horticulture. 1-12 April 2019. 4 hours.
3. Study Abroad Summer Courses Ual. 2019. SUSTAINABLE PROTECTED HORTICULTURE: SOILLESS CROP SYSTEMS AND HYDROPONICS. 1-25 July 2019. 10 hours.

Congress communications and courses

1. Communication to Congress. Title of the work: Evaluation of silicon in the nutrient solution with fertigation in cucumber, melon and pepper crops.
fertigation in cucumber, melon and pepper crops. Authors: Miguel Urrestarazu Gavilán; Francisca Carrillo Ferrón; Jose alan huerta trujillo; Osmar Jair Ortiz Pedraza; Abril Anahi Rojas López. Name of the congress: XXIII Verano de Investigación Científica y Tecnológica del Pacífico 2018. Place and date of celebration: Nuevo Vallarta, Nayarit, (15/08/2018).

2. Communication to Congress. Title of the work: Implementation of algorithms in MATLAB for the measurement of roots. the measurement of roots. Authors: Miguel Urrestarazu Gavilán; Francisca Carrillo Ferrón; Juan Carlos Gómez, Julio César Regalado. Name of the congress: I Congreso Jóvenes Investigadores en Ciencias Agroalimentarias. Agroalimentary Sciences. Place and date: University of Almería, (20/12/2018).

3. Symposium Communication. Title of the work: Implementation of algorithms in MATLAB for the measurement of roots. the measurement of roots. Authors: Francisca Carrillo Ferrón; Miguel Urrestarazu Gavilán; Juan Carlos Gómez; Julio César Regalado. Name of the congress: VII Simposio de investigación en Ciencias Experimentales. Sciences. Place and date: Almería, Spain. (15/11/2018).

4. Conference communication: LETTUCE MICROGREENS: EFFECT OF ARTIFICIAL LIGHTING LEDS ON THE GROWTH AND CAROTENOIDS CONTENT IN. Authors: aFerrón-Carrillo, F., aGuil-Guerrero, J.L.*, bLyashenko, S., aGonzález-Fernández, M.J., aUrrestarazu, M. Conference name: EuroFed Lipid Congress Seville 2019. Status: Accepted. Date and Place Date and Place: Seville, Spain, October 2019.

5. Conference Communication. Title of the work: Fatty Acids, Tocopherols and Sterols from 50 Ribes Taxa. Authors: aLyashenko S., bGonzález-Fernández M.J., bFerrón-Carrillo F., bFabrikov D., bUrrestarazu M., bGuil-Guerrero J.L.*Congress name: EuroFed Lipid Seville Venue and date: Seville, Spain (20/10/2019).

6. Conference Communication. Title: Highly concentrated ARA, DHA and EPA by urea complexation methodology. urea complexation methodology Authors: M.J. González-Fernández1*,

S. Lyashenko1, F. Ferrón- Carrillo1, J.L. Guil-Guerrero1Conference name: EuroFed Lipid Seville Venue and date: Seville, Spain (20/10/2010). Seville, Spain (20/10/2019).

7. Conference Communication. Title: Argan oil: influence of different extraction systems on fatty acid profiles and oil systems on fatty acid profiles and oil quality Authors: M.J. González-Fernández1*, S. Lyashenko1, F. Ferrón-Carrillo1, J.L. Guil-Guerrero1. Conference name: EuroFed Lipid Seville Venue and date: Seville, Spain (20/10/10). Seville, Spain (20/10/2019).

8. Conference Communication. Title of the work: Phenolic compounds from Borago species.
Authors: aLyashenko S., bFabrikov D., bGonzález-Fernández M.J., bFerrón-Carrillo F., bUrrestarazu M., bGuil-Guerrero J.L.*Congress name: EuroFed Lipid Seville Venue and date:
Seville, Spain (20/10/2019).

9. Conference Communication. Title of the work: Assessing the n-3 PUFA status of Palaeolithic hominins trough faunal assemblages Authors: José Luis Guil-Guerrero1*, María José González-Fernández1, Dmitri Fabrikov1, Svetlana Lyashenko2, Francisca Ferrón-Carrillo1, Miguel Urrestarazu1. Name of the congress: EuroFed Lipid Seville Place and date of celebration: Seville, Spain (20/10/2019).

10. Conference Communication. Title of the work: Implementation Phenolic compounds from Borago species. Authors: aLyashenko S., bFabrikov D., bGonzález-Fernández M.J., bFerrón-Carrillo F., bUrrestarazu M., bGuil-Guerrero J.L.*Congress name: II Congreso Jóvenes Investigadores en Ciencias Agroalimentarias. en Ciencias Agroalimentarias. Place and date: University of Almería, (20/10/2019).

Courses

1. Training Network Courses 2017. University of Almeria. Advanced techniques in greenhouse greenhouse technology. (27/11/2017-01/12/2017), 40 hours.

2. Study Abroad Summer Program University of Almeria. Sustainable Protected Horticulture. (02/07/2018-26/07/2018), 80 hours.

3. XVIII UAL Summer Courses. Smart Biohouses: Business trends in the agri-food sector. agri-food sector. (10/07/2017-12/07/2017), 25 hours.

4. SCOPUS advanced level classroom training course UAL. (16/10/2017), 3 hours.

5. Entrepreneurship project preparation for the Open Future UAL Alumni Scholarship 2016/17, 20 hours.

6. Fruit and vegetable research and development in the Almeria countryside. Openresearchers. Commission European Commission in the Marie Skolodowska-Curie Actions call. University of Almeria. (29/09/2017), 20 hours.

7. I Ciaimbital UAL Transfer Conference. (5/10/2017-6/10/2017), 19 hours.

8. Conference attendance: Name of the conference: El Innovadero. Innovation and agri-food.
Place and date of the congress: Almería, Spain. 25 hours (27/03/2019-28/03/2019).
13. Attendance at a talk: Name of the talk: "MANAGEMENT OF TOXIC AND HAZARDOUS WASTE OF CHEMICAL AND BIOLOGICAL HAZARDOUS WASTE OF CHEMICAL AND BIOLOGICAL ORIGIN". Place and date: Almería, Spain. 6 hours (05/12/2018).

9. Dissemination and scientific communication. UAL Summer Courses 2019. (01/07/2019-03/07/2019). 25 hours.

10. Autumn Science Days Ual 2019. 24-26 September 2019. International Doctoral School. University of Almeria. 17 hours.

Work experience and languages

1. Research work at the UAL-ANECOOP Experimental Farm, for the implementation of the UNE ISO/IEC 17020 and 17025 the UNE ISO/IEC 17020 and 17025 standards in the laboratories of the farm, and as an advisor for the treatment of diseases and pests to treatment of diseases and pests to the research groups of the UAL. Researcher at CIAIMBITAL to help in the collection of information for the implementation of European and international projects in which the University of Almeria participates. and international projects in which the University of Almeria participates, (2017-2018).

2. Research work in the Food Technology laboratory of the University of Almeria, lipids, carbohydrates, carbohydrates, carbohydrates, carbohydrates
Almeria, lipids, carotenes, phenols, HPLC liquid chromatography, gas chromatography, cell culture. cell culture. (2018-2021).

3. Work in private company Infoagro Systems S.L. Director of the agronomy and research department. (04/11/2019-03/10/2020).

4. Winner of the Telefónica Open Future Start Up 2017 Grant: "PROPOSAL R+D+i FOR

THE IMPLEMENTATION OF AN INTERPRETATIVE CENTRE FOR THE

SUSTAINABLE PRODUCTION OF DEHYDRATED FRUITS AND VEGETABLES FROM THE DEHYDRATED FROM THE ALMERIA REGION".

5. Agri-food Innovation Awards of GRUPO LA CAÑA II and III edition. Category student finalist (2018-2019).

6. ENGLISH LEVEL C1 CAMBRIDGE.