

Algorithm implementation in MATLAB for root measurement

^aFerrón-Carrillo, F, ^bGómez-Cortés, JC, ^bRegalado-Sánchez, J, ^aUrrestarazu, M, ^aNovas Castellano, N*.

*^aCIAIMBITAL, University of Almería, Almería, Spain

^bTechnological Institute of Celaya, Guanajuato, México

*Corresponding author: nnovas@ual.es

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.

Keywords: Thermography; Non-invasive method; Image; Soilless culture.

Copyright statement: ©2020. This manuscript version is made available under the CC-BY-NC-ND 4.0 license <https://creativecommons.org/licenses/bync-nd/4.0/> Published as: Francisca Ferrón-Carrillo, Juan Carlos Gómez-Cortés, Julio Regalado-Sánchez, Miguel Urrestarazu, Nuria Novas Castellano, Algorithm implementation in MATLAB for root measurement, Computers and Electronics in Agriculture, Volume 174, 2020, 105487, ISSN 0168-1699, <https://doi.org/10.1016/j.compag.2020.105487>.

Highlights

A better efficiency of root analysis was found with a non-invasive method.

MATLAB produced a smaller error when calculating volume conversion than Image J.

Root area was recorded with an error of less than 5% between the results obtained.

The application of the method in hydroponics could be extended in soil crops.

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 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 x 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 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, ϵ , 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 hours 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 centimetres (cm), the frontal photograph, the back photograph, and a distant photograph were obtained (Figure 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 $\pm 1.8^{\circ}\text{C}$.

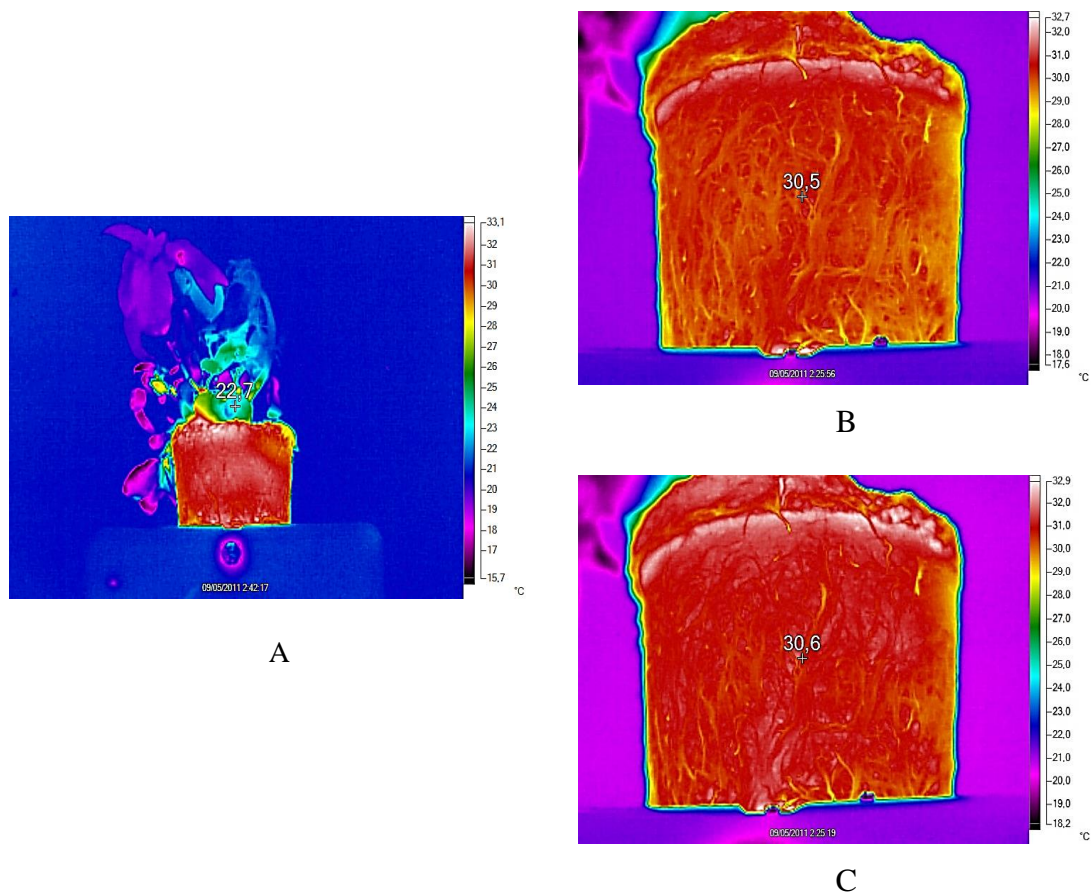


Figure 1. Thermography images: A. (far front), B. (close front) and C. (close back) of a pot under their temperature gradient.

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 hours 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 personalized scanning, analysis and processing using the editor included in ImageJ, as can be seen in Figure 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 Figure 2, proceeded as follows:

1. Improve the image (step 1 of Figure 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 microns) 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 Figure 3. The processing was done in 5 steps obtaining finally an image to measure the area of the photographed roots.

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.

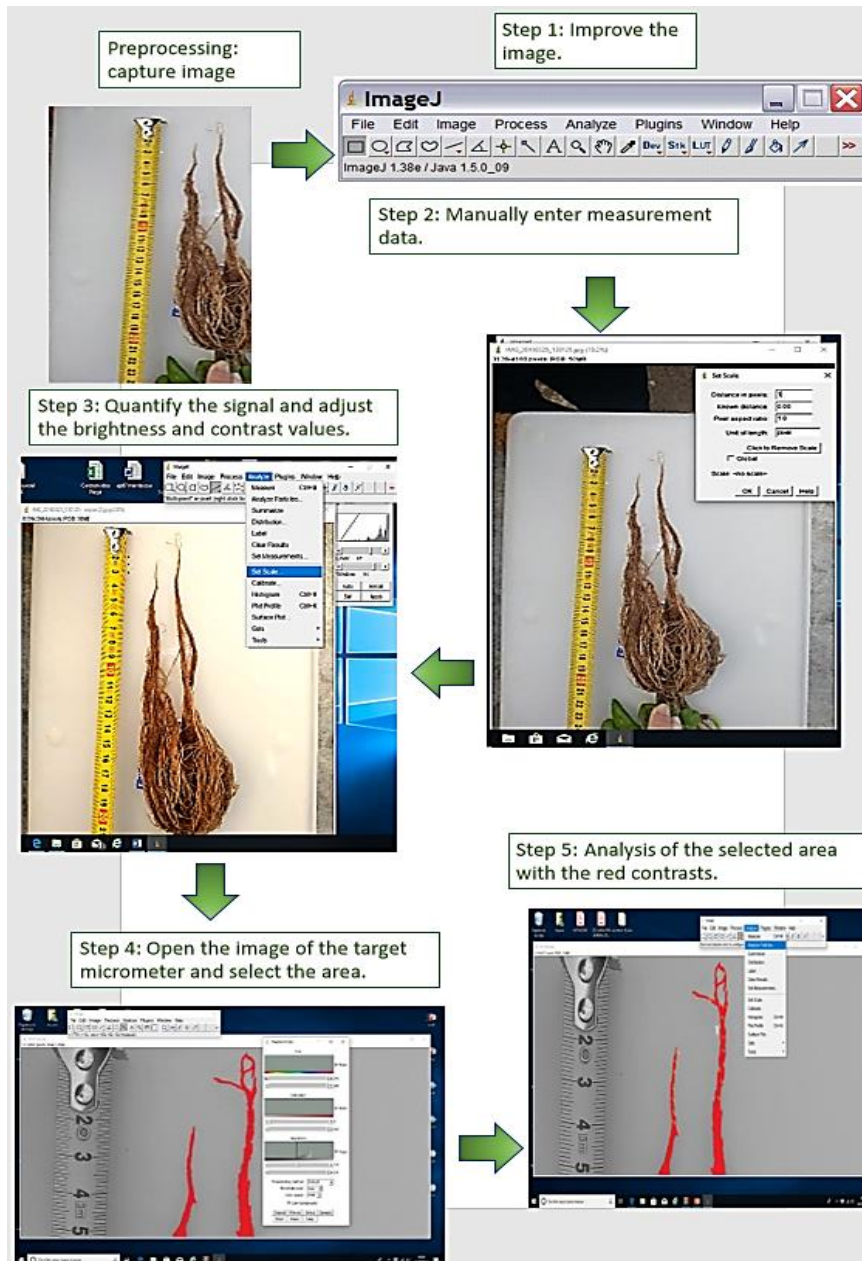


Figure 2. Root analysis by Image J.

Syntax

$$I = \text{rgb2gray}(\text{filename.jpg})$$

$$\text{newmap} = \text{rgb2gray}(I)$$

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

Function $f: E \rightarrow R$ being a grayscale image, assigning points from a Euclidean space or a discrete E grid (such as R^2 or Z^2) 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:

$$T_w(f) = f - f \circ b$$

Where the number indicated the opening operation.

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

$$T_b(f) = f \bullet b - f$$

$$T_b(f) = f \bullet b - f$$

Where \bullet 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

$$J = \text{imtophat}(I, SE)$$

$$J = \text{imtophat}(I, \text{nhood})$$

4. *Binarize*. `BW = imbinarize(I)` created a binary image from the 2D or 3D I grayscale image by replacing all values above a globally determined threshold by 1s and setting all other values to 0s. By default, `imbinarize` uses Otsu's method, which chose the threshold value to minimize the intraclass variance of the black and white threshold pixels.

Syntax

$$BW = \text{imbinarize}(I)$$

Filters were required to reduce noise:

- I. Erosion of a binary image f by structuring elements, s (denoted s) produced a new binary image $g = f \circ s$ 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.: $g(x, y) = 1$ if s is set to f and 0 otherwise, repeating for all pixel coordinates (x, y) .

Erosion with small square structuring elements (e.g. 2 x 2 to 5 x 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 $g = f \oplus s$ 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:

$$f = d_{\text{image}} / d_{\text{benchmark}} \quad (1)$$

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.

$$\text{diameter}_{\text{final}} = \text{diameter}_{\text{initial}} (\text{mm}) / f \quad (2)$$

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).

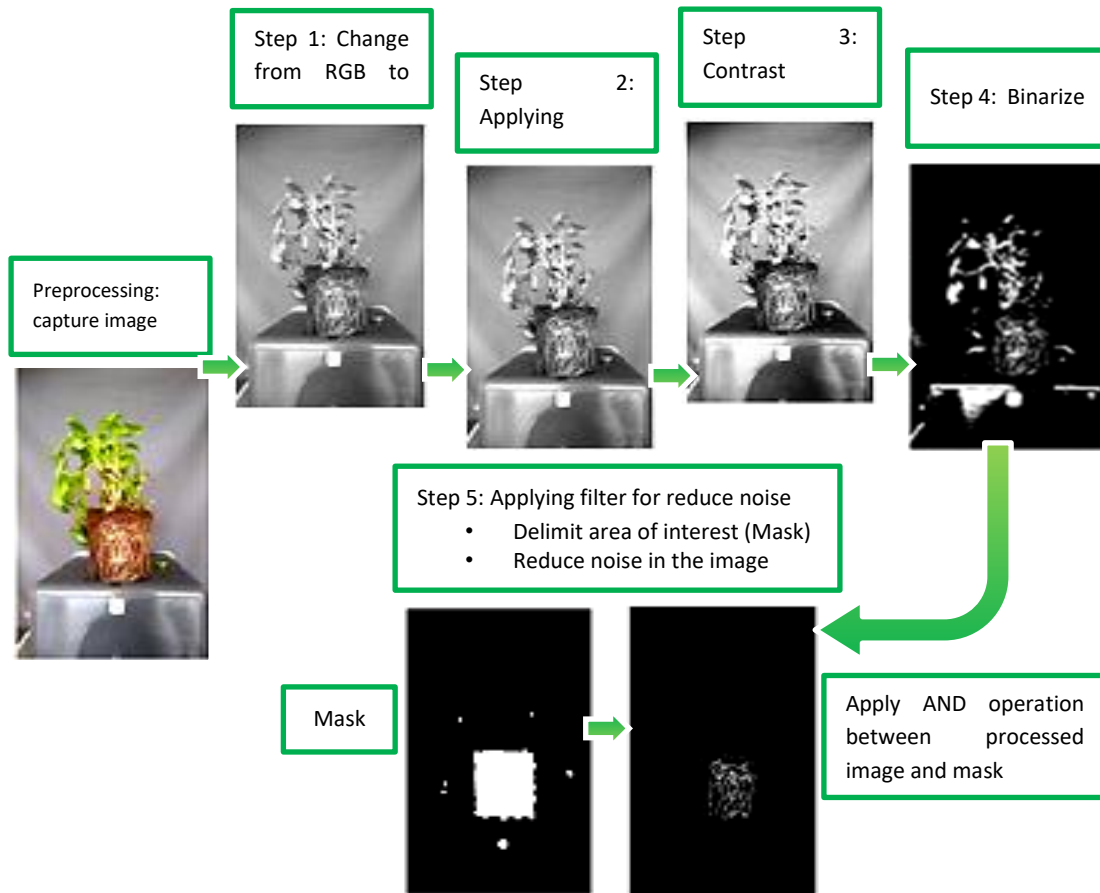


Figure 3. Root analysis by MATLAB.

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 (Figure 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 Figure 4, the volume did not exceed $100 \text{ ml plant}^{-1}$ with the exception of two of the units

tested (M2), at 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 Figure 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.

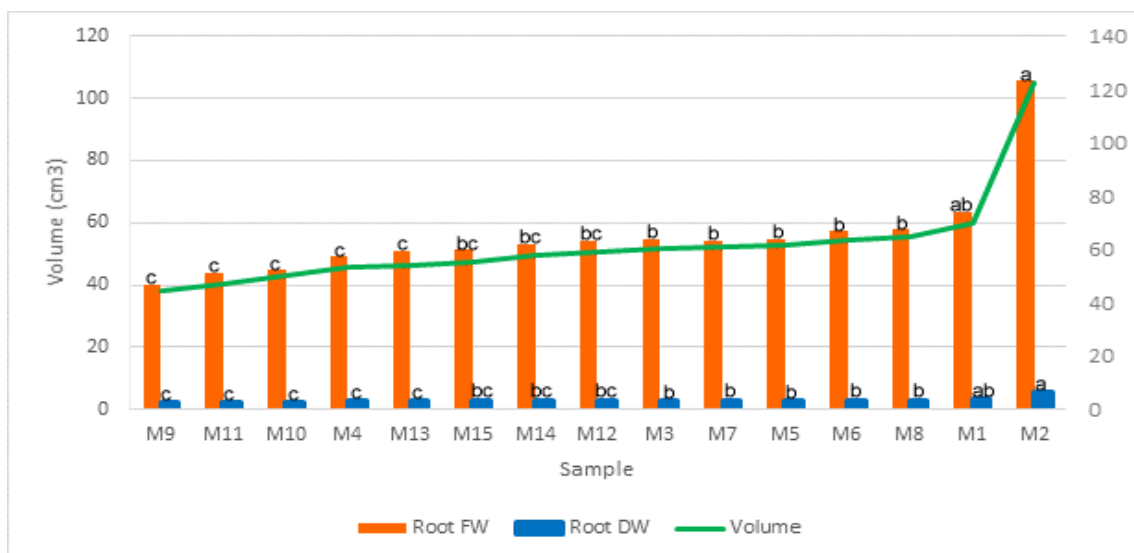


Figure 4. Comparative graph of the fresh and dry weight of the root with the volume of each plant. Different letters indicate significant differences between vegetative parameters of fresh and dry weight in a Tukey Test at $P < 0.05$

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.

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 $P < 0.05$.

Samples	Measurements		Fresh weight			Dry weight		
	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 _{cd}	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 _{cd}
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	47,00 _{bc}	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

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

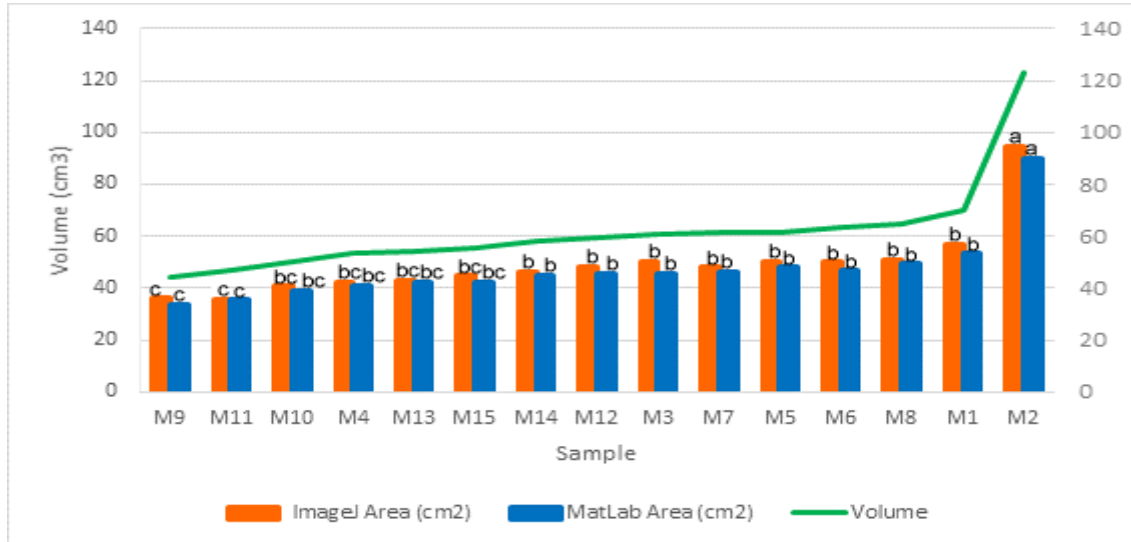


Figure 5. Comparative graph of the analysis by Image J and analysis by MATLAB with the volume of each plant. Different letters indicate significant differences between ImageJ area and MATLAB area in a Tukey Test at $P \leq 0.05$.

Figure 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 Figure 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. Figure 7 presented the averages with their coefficients, obtaining the following values for each coefficient:

$$A_{ImageJ} = 1.2495$$

$$A_{MatLab} = 1.3094$$

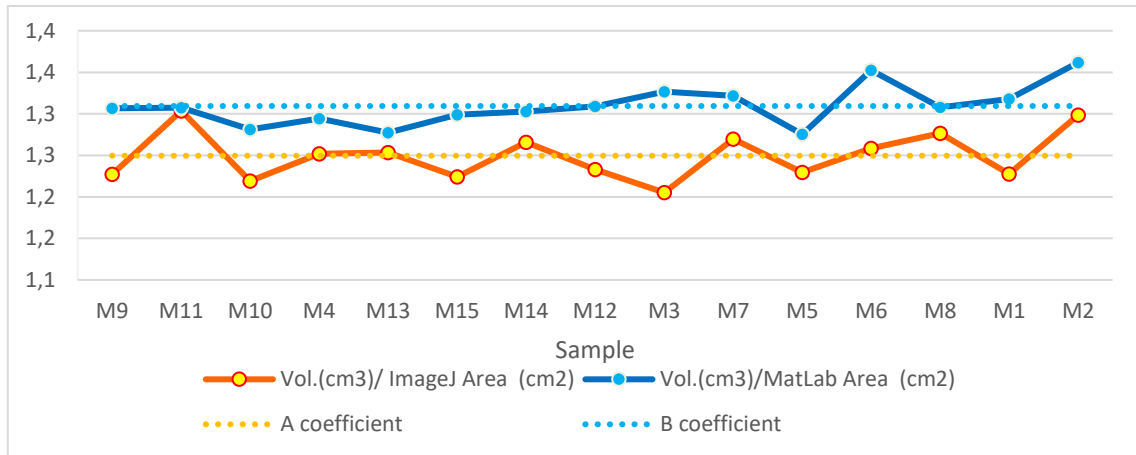


Figure 6. Comparative graph of the equivalence in cm between Vol.(cm³)/Area (cm²).

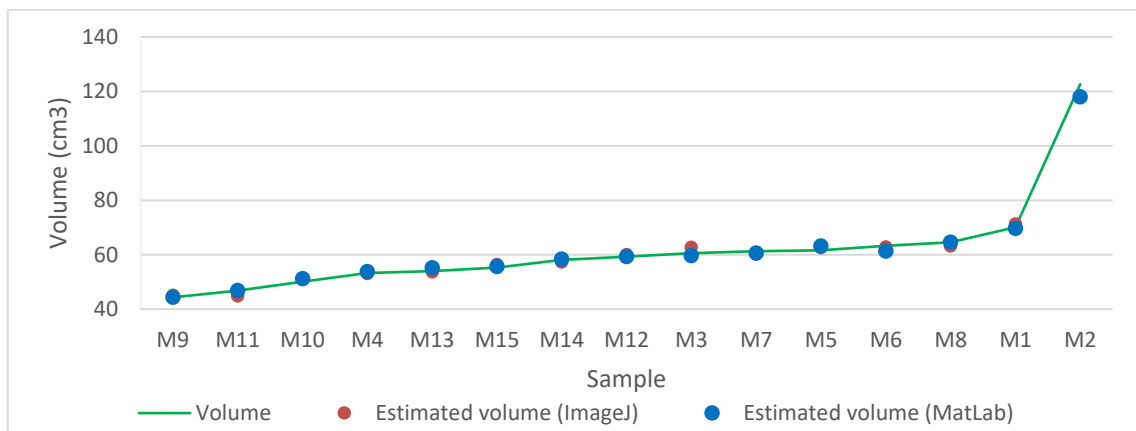


Figure 7. Distribution of estimated volume value.

Figure 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 equation (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.

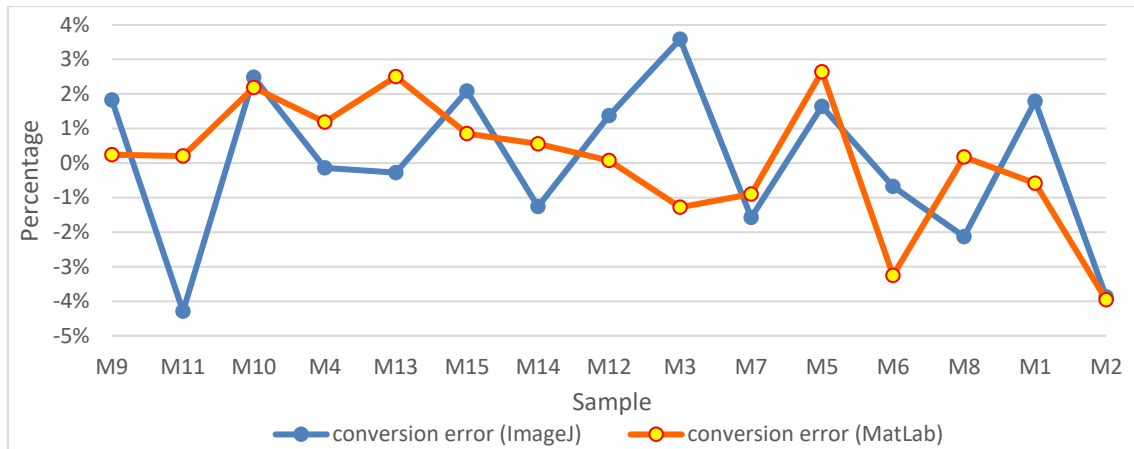


Figure 8. Comparative graph of the errors made from conversion to volume.

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 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 IvÑ, 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 Figure 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 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 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 postprocessing 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.

Acknowledgements

Thanks to the CIAIMBITAL working group and the TIC019 Research group of the University of Almería (Spain) for their subsidy.

References

1. Y. Altmann, S. Mc Laughlin, M.J. Padgett, V. Goyal, A. Hero, D. Faccio. 2018. Computational Imaging. Science. 361:6403. Doi: 10.1126/science.aat2298.
2. R. Callejas-Rodríguez, E. Rojo-Torres, C. Benavidez-Zabala, E. Kania-Kuhl. 2012. Crecimiento y distribución de raíces y su relación con el potencial productivo de parrales de Vides de Mesa. *Agrociencia*, 46(1):23–35.
3. D. Chen, E.C. Yang, J. Jiang, T.T. Lin. 2012. An imaging system for monitoring the in-and-out activity of honey bees. *Computer Electronics in Agriculture*. 89:100–109. 10.1016/j.compag.2012.08.006.
4. M.T. Domínguez, Marañón, T, Murillo, J.M., Hidalgo, J.A., Madejón, P. 2019. Crecimiento y morfología foliar de especies leñosas en las reforestaciones del Corredor Verde del Guadiamar (Sevilla). *Cuadernos de la Sociedad Española de Ciencias Forestales*, ISSN. 20:1575–2410.
5. Y. Fang, R.P. Ramasamy. 2015. Current and prospective methods for plant disease detection. *Biosensors* 5:537-561. doi:10.3390/bios5030537.
6. P. García-Caparrós, A. Llanderal, J. Rodríguez Ortiz, I. Maksimovic, M. Urrestarazu, M. Lao. 2018. Rosemary growth and nutrient balance: Leachate fertigation with leachates versus conventional fertigation. *Sci Hortic*. 242:62-68. Doi:10.1016/j.scienta.2018.07.024.
7. A.M. González. 2018. ImageJ: una herramienta indispensable para medir el mundo biológico. *Sociedad Argentina de Botánica; Folium*; 1; 9:1-17.
8. R. Ishimwe, K. Abutaleb, F. Ahmed. 2014. Applications of thermal imaging in agriculture—A review. *Advances in Remote Sensing* 3:128-140. Doi:10.4236/ars.2014.33011.
9. D. Jerez Mompies, R. Martín. 2012. Comportamiento del crecimiento y el rendimiento de la variedad de papa (*Solanum tuberosum* L.) Spunta. *Cultivos Tropicales*, 33(4):53–58.
10. M. Lukac, D.L. Godbold. 2001. A modification of the ingrowth core method to determine root production in fast growing tree species. *Journal of Plant Nutrition and Soil Science* 164:613–614.

11. Mathworks. 2018. Global Optimization Toolbox: User's Guide (r2018b). Retrieved November 10, 2019 from www.mathworks.com/help/pdf_doc/gads/gads_tb.pdf
12. L. McAusland, P. Davey, N. Kanwal, N. Baker, T. Lawson. 2013. A novel system for spatial and temporal imaging of intrinsic plant water use efficiency. *Journal of Experimental Botany*. 64:4993–5007.
13. N. Novas, J.A. Gázquez, J. MacLennan, R.M. García, M. Fernández-Ros, Manzano-Agugliaro, F. 2017. A real-time underground environment monitoring system for sustainable tourism of caves. *Journal of Cleaner Production*. 142:2707–2721. Doi: 10.1016/j.jclepro.2016.11.005.
14. M.R. Ortega, C. Ivã. 2012. Hydroponic and aquaponic production of sweet basil (*Ocimum basilicum*) and giant river prawn (*Macrobrachium rosenbergii*). *Trop and Subtrop Agroecosyst*. 15 (S2):63–71.
15. J. Rahkonen, H. Jokela. 2003. Infrared radiometry for measuring plant leaf temperature during thermal weed control treatment. *Biosystems Engineering*. 36(3):257–266.
16. Z. Sanquin, Z. Haonan, C. Mingmei, C. Xicun, L. Yutao. 2019. Rapid yield prediction in paddy fields based on 2D image modelling of rice panicles. *Computers and Electronics in Agriculture*. 162:759–766.
17. C.A. Schneider, W.S. Rasband, K.W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature methods*. 9(7):671–675. PMID 22930834.
18. C. Sonneveld, A.L. Van den Bos, W. Vooght. 2011. Modeling osmotic salinity effects on yield characteristic of substrate grown greenhouse crops. *Journal of Plant Nutrition*. 27:1931–1951.
19. M. Urrestarazu, V. Gallegos, J. Álvaro. 2017. The Use of Thermography Images in the Description of the Humidification Bulb in Soilless Culture. Doi:10.1080/00103624.2017.1374399.
20. S.K. Vidyarthi, H.M. El Mashad, R. Khir, R. Zhang, R. Tiwari, Z. Pan. 2019. Evaluation of selected electric infrared emitters for tomato peeling. *Biosystems Engineering*. 184:90–100. Doi:10.1016/j.biosystemseng.2019.06.006.
21. Warrenton. 2018. Statgraphics Centurion XVIII. Statgraphics Technologies, Inc. The Plains, Virginia.