Mycelial growth assessment by digital image analysis in R software environment

Evaluación del crecimiento del micelio mediante análisis de imagen digital en R

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ABSTRACT

This study was aimed at developing a script in R to assess fungal growth in Petri dishes using computer vision. The script was developed to aid studies in agricultural phytopathology. The fungi used were *Elsinoe ampelina*, *Fusarium oxysporum* and *Fusarium verticillioides*. The images were analyzed with R and the EBImage package. The command computeFeatures.shape was used to calculate the area and the mean diameter of the colony and the label in square pixels. The script was run in a loop to automate the analysis of all images in sequence. The script developed in R with the package EBImage was able to recognize and measure the fungus colonies examined. Simultaneously with photographing, the mean diameter of colonies was obtained by calculating the arithmetic mean of the diameter measured with two straight perpendicular lines using a ruler. Using the mean diameter, the area of the colony was calculated as a circle. The coefficient of determination and the root mean square error were calculated using the measurements and the values obtained with the program. Based on the coefficient of determination, the measurements obtained with the software are similar to those made using a ruler. The script reduces the image analysis time and increases accuracy, especially for non-uniform colonies.

Key words: software, computer vision, EBImage, mycelial growth.

RESUMEN

Este estudio tuvo como objetivo el desarrollo de un script en R para evaluar el crecimiento de hongos en placas de Petri utilizando la visión por ordenador. El script fue desarrollado para ayudar a los estudios de fitopatología agrícola. Los hongos utilizados fueron Elsinoe ampelina, Fusarium oxysporum *y* Fusarium verticillioides. *Las imágenes se analizaron a continuación con R y EBImage. La función computeFeatures.shape se utilizó para calcular el área y el diámetro medio de la colonia y de la etiqueta en píxeles cuadrados. La secuencia de comandos se ejecuta en un bucle para automatizar el análisis de todas las imágenes en secuencia. El script desarrollado en R con EBImage era capaz de reconocer y medir las colonias de hongos examinadas. Simultáneamente a fotografiar, el diámetro medio de las colonias se obtuvo mediante el cálculo de la media aritmética del diámetro medido con dos líneas perpendiculares rectas utilizando una regla. Utilizando el diámetro medio, el área de la colonia se calculó como un círculo. El coeficiente de determinación y el error cuadrado medio de la raíz se calcularon utilizando las medidas y los valores obtenidos con el programa. Con base en el coeficiente de determinación, las mediciones obtenidas con el software son similares a las realizadas utilizando una regla. La secuencia de comandos reduce el tiempo de análisis de imágenes y aumenta la precisión, especialmente para colonias no uniformes.*

Palabras clave: software, visión por computador, EBImage, crecimiento del micélio.

Introduction

Computer vision is a technology that can extract useful information by processing and manipulating images in a computer (Wangenheim & Comunello, 2005). It has been used to detect and quantify plant diseases and its greatest advantage is being quick, non-destructive and objective (Barbedo, 2013).

According to the literature, software programs have been developed to quantify plant diseases, such as QUANT (Vale *et al.*, 2003) that processes, segments, and measures digital images. The thousands of colors in a digital image are reduced to three or four colors, each representing objects in the image; background, and healthy, chlorotic and necrotic portions.

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The program ImageJ is of special interest as it involves several areas of science, not only phytopathology (Abràmoff *et al.*, 2004). It allows the development of macros and the analysis of a large number of images simultaneously. In addition, measurements of images and arithmetic and logical operations among images can be carried out. Other programs are also available such as Citrusvis (Pazoti *et al.*, 2006), AFSoft (Jorge & Silva, 2009), and Image Tool (Wilcox *et al.*, 2002). These programs interpret and process digital images until a solution for a given problem is found.

The R language was originally created for statistical calculations and graphs (Ihaka & Gentleman, 1996). However, its applications can be extended with additional packages. The package EBImage is a tool for reading, writing, processing and analyzing digital images. It also allows the automation of tasks in the R environment. The package examines digital images using the RBG color system, and recognizes colors based on pixel intensity, since images can be manipulated using algebraic operations such as sum, product, comparison and convolution (Pau *et al.*, 2010).

This study was aimed at developing a script in R to assess fungal growth in Petri dishes using computer vision. The script was developed to aid studies in agricultural phytopathology.

Material and Methods

The study was carried out at the Laboratory of Phytopathology of UFSC, Curitibanos Campus. The fungi used were *Elsinoe ampelina*, the causal agent of grape anthracnose; *Fusarium oxysporum*, which causes Fusarium rot in garlic; and *Fusarium verticillioides*, the causal agent of Fusarium ear rot in maize.

A disk of mycelium of 5 mm diameter was placed in the center of a Petri dish containing PDA medium (Potato-Dextrose-Agar). The Petri dishes were sealed and incubated in a growth chamber at a temperature of 25 ± 2 ºC in the dark. Petri dishes with fungi were numbered for identification.

A mini-studio was set up in the same laboratory, which consisted of a Nikon Coolpix P520 digital camera mounted on a tripod. Images of Petri dishes with mycelia were obtained against a black background and identified with a paper label of known area of 4 cm^2 (2 x 2 cm). Photographs were taken daily for seven days, with five repetitions for each fungus, totaling 105 images. The images were then analyzed with R and the EBImage package (Pau *et al.*, 2010). This package transforms images into numerical matrices by manipulating them using geometric and algebraic tools that allow the identification and measurement of the objects.

Images were first resized to 800 pixels, normalized and gamma corrected to decrease the brightness and increase the contrast between the colony and the Petri dish. Based on the central coordinates of the image, the command *floodFill* was used to fill all similar pixels of the fungus colony on the Petri dish, and then transformed into a binary image where white represented the fungus colony and black the background. The commands *fillHull* (to eliminate black dots inside the white area), erode and dilate were used to eliminate image noise. The same procedure was used to highlight the label with known area. The command *computeFeatures. shape* was used to calculate the area and the mean diameter of the colony and the label in square pixels. To transform the area into square centimeters, the following equation based on the label with an area of 4 cm^2 was used:

$$Ac_{cm^2} = \frac{Ac_{pix}}{Ae_{pix \cdot 4_{cm^2}}}$$

Where

Ac_{cm2} = Area of the colony in square centimeters
Ac_{pix} = Area of the colony in square pixels
Ae_{pix} = Area of the label in square pixels

The script was run in a loop to automate the analysis of all images in sequence. The images that were not recognized were edited with the software GIMP 2 and reanalyzed. Simultaneously with photographing, the mean diameter of colonies was obtained by calculating the arithmetic mean of the diameter measured with two straight perpendicular lines using a ruler. Using the mean diameter, the area of the colony was calculated as a circle. The coefficient of determination and the root mean square error were calculated using the measurements and the values obtained with the program.

Results and Discussion

The original images were manipulated with the package EBImage of the R software to identify fungus colonies (Figure 1C and 1D) and the label
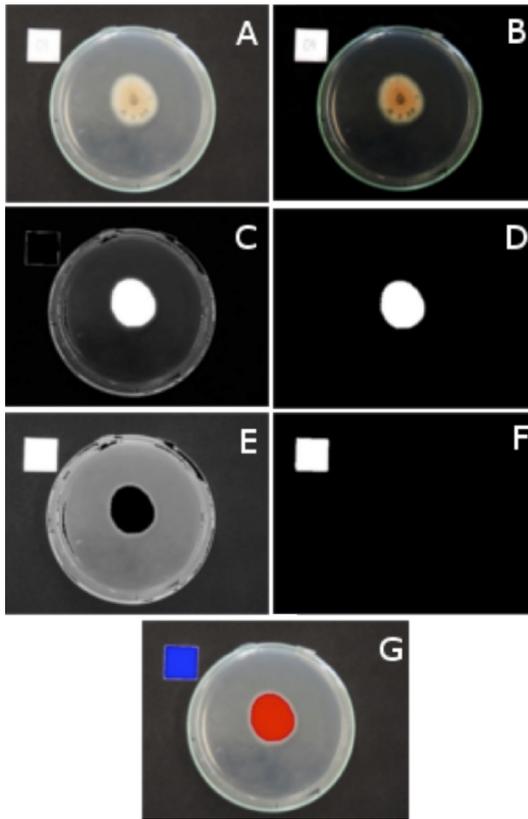
Figure 1. Original image (A), Gamma corrected image (B), Binary image showing the fungus colony (C, D), Binary image showing the label (E, F), Image with the identified colony and label (G).

The morphological characterization of fungi was studied mainly with applications in industrial fermentation processes. Paul & Thomas (1998) described four applications of image analysis systems in the growth assessment of filamentous microorganisms used in industrial fermentation processes. These authors observed that the mycelial growth of these microorganisms is complex and that computer vision systems can be useful to model mycelial growth. Treskatis *et al.* (1997) developed an algorithm for the morphological characterization and estimation of biomass of filamentous microorganisms by means of digital image analysis that allowed continuous monitoring of the fermentation process. Couri *et al.* (2006) developed a computer vision system that measured the area occupied by the hyphae of fungi and correlated it with the total biomass produced. As shown in Figure 2, the measurements obtained by the program were similar to those calculated using traditional methods to assess *Elsinoe ampelina* ($R^2 = 0.9798$), *Fusarium oxysporum* ($R^2 = 0.9600$), and *Fusarium verticillioides* ($R^2 = 0.9243$). Thus the dependent variable may be explained by the independent variable in the linear model. Using the statistical variables, the root mean square error was calculated for *Elsinoe ampelina* (1.66), *Fusarium oxysporum* (3.16), and *Fusarium verticillioides* (5.24).

Conclusions

The script developed in R with the package EBImage was able to recognize and measure the fungus colonies examined. The script reduces the image analysis time and increases accuracy, especially for non-uniform colonies. Based on the coefficient of determination, the measurements obtained with the software are similar to those made using a ruler.

of known area (Figures 1E and 1F). As observed in Figure 1B, the gamma correction applied to the image is important to enhance the contrast between the object of interest and the background. In figure 1D, the area of the colony is completely isolated from the remaining image. The same is observed for the label of known area (Figure 1F).
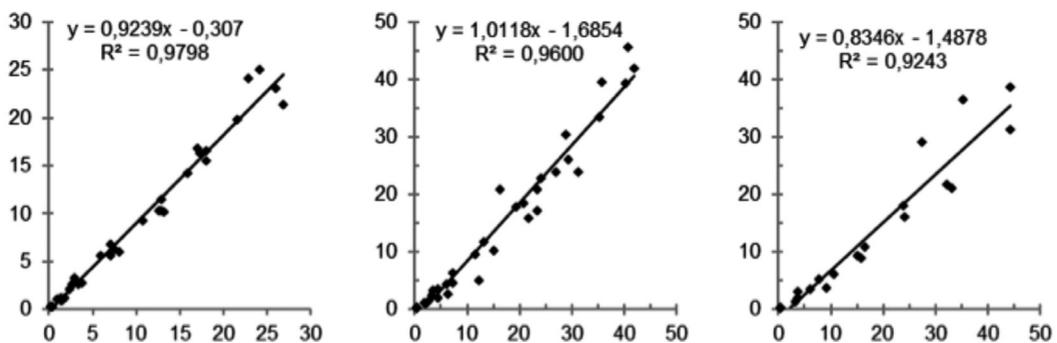

Figure 2. Area of the colony calculated by ruler measurements (AR) and obtained with the script (AS). *Elsinoe ampelina* (A), *Fusarium oxysporum* (B) and *Fusarium verticillioides* (C)

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