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Arabidopsis Growth Analysis Using Image Processing Technology

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Abstract: This paper describes the use of image processing technology on the measurement of Arabidopsis leaf areas. Firstly, a black and white checkerboard was used to correct the image. This was done by finding the corner of the grid and performing affine transformation. The corners were also used to calculate the number of pixels of the checkerboard grid. Secondly, the plants were segmented with the values of the red, green, blue components of each pixel. Finally, the areas were calculated with the number of the pixels of the plants and the checkerboard grid in the image, as well as the real area of the board grid. The result has shown that this non-destructive method can reflect the growth rate by measuring the areas of the Arabidopsis leaves.

Introduction

Arabidopsis is an important research object of genectics. Although its gene sequence has been completed since 2000[1], people still cannot get a better understanding of some gene function. Nowadays, the phenotype genomics has attracted more and more attention. Phenotype means the biological traits characteristic of organisms under the combined effects of genetic and environmental factors. The previous study shows that even a small variation in the genome of the plants can result in different phenotypes. In addition, if the environmental factors are different, the phenotypes of the plants can also vary. For example, the phenotypes of the Arabidopsis during their growth process were different when they were grown in different culture media[2]. Therefore, the analysis of plant phenotypes can fully illustrate the physiological functions, as well as the impacts of different environmental conditions to the growth. With the results, we can select the gene and environment condition, which are beneficial for the plants, providing a more efficient means in the cultivation of new crops. In summary, the detection and analysis of the phenotypic characteristics of Arabidopsis has great significance.

This paper will focus on the area measurement of Arabidopsis leaves. Leaf area is closely related to its ecological processes, including photosynthesis, transpiration and energy balance. The botanist has proven that the leaf area is a very essential parameter in the estimation of growth rate and yield [3]. There are many methods to measure leaf area. One way is to destructively measure the parameter, such as squares method [4]. Some researchers [5,6] used scanner to obtain the images of leaves, then calculated the area with image processing software. The advantage is that the result obtained is very accurate. However, the disadvantage of this approach is also obvious that this method only works for small quantities of the measured object. Some other researchers [7] obtained

the images with camera. They put the object in a large rectangle as reference in advance. When calculating the area, they inputted the endpoints of the rectangle manually, which would result in some error.

In this paper, we will use a black and white checkerboard as reference, thus we can detect the region automatically. Compared with the destructive method, this non-destructive method can be carried out for continuous measurements.

Materials and Methods

Materials. About 150 images of five kinds of Arabidopsis were taken with natural backgrounds. The difference between those Arabidopsis was their quantity of AMT1.1 gene. The gene plays an important role in their NH_4^+ acquisition. The first kind was named as "b", with over-expression of AMT1.1 gene. The second kind "p" was similar to "b". Their difference existed in the gene vectors. The third kind "a" is wild type with empty vector. The fourth kind "w" is wild type, while the fifth kind "m" is mutant without AMT1.1 gene.

The images were taken every two or three days from 13 August to 22 August, 2012. Each pot had five Arabidopsis. A 2.5cm×2.5cm black and white checkerboard was put in the pot to be reference. Each grid is 5mm×5mm. One of the original images is shown in Fig.1(a).

Corner acquisition. Corner is an important local feature of an image. It defines the points with large gray-scale variation in at least two directions. Corner is easy to measure and is able to adapt to changes in ambient light. The most common algorithm to detect corner is Harris corner detections algorithm [8]. It has many advantages. First of all, it only needs to compute the first-order differential of the gray value and filtering, making the operation simple. Secondly, it can always select the most useful feature points, rather than those in the texture region because of its window operation. Finally, it has high stability and robustness, and can choose the number of points extracted. In this paper, we used the algorithm to detect the corners of black and white checkerboard. To find the corner, the first derivatives of the horizontal (I_x) and vertical direction (I_y) of each pixel were computed. Then we got three images: I_x^2, I_y^2 and their product I_{xy}. Taking into account the effects of noise in the image under normal circumstances, they were filtered to remove noise points with Gaussian filter. The corner was detected with the following function:

$$R(i,j)=\det(M)-0.06*trace(M)^2 \tag{1}$$

$$\text{Where } M=\begin{bmatrix} I_x^2(i,j) & I_{xy}(i,j) \\ I_{xy}(i,j) & I_y^2(i,j) \end{bmatrix} \tag{2}$$

We could obtain the maximum of R_{max}. If $R(i,j)>0.01*R_{max}$ as well as it is the local maximum in the 3×3 neighborhood, then it is the corner point.

Image correction. It is inevitable that the image we obtained has some geometric distortion. For two images, their plane projective transformation can be expressed as:

$$\begin{bmatrix} x_1' \\ x_2' \\ x_3' \end{bmatrix}=\begin{bmatrix} h_{11} & h_{12} & h_{13} \\ h_{21} & h_{22} & h_{23} \\ h_{31} & h_{32} & h_{33} \end{bmatrix}\begin{bmatrix} x_1 \\ x_2 \\ x_3 \end{bmatrix} \tag{3}$$

where $[x_1 \quad x_2 \quad x_3]$ is the homogeneous coordinates of the original image, where $[x_1' \quad x_2' \quad x_3']$ is the homogeneous coordinates of the distorted image. Their non-homogeneous coordinates are given as:

$$\begin{cases} x = \dfrac{x_1}{x_3} \\ y = \dfrac{x_2}{x_3} \end{cases} \quad \begin{cases} x' = \dfrac{x_1'}{x_3'} \\ y' = \dfrac{x_2'}{x_3'} \end{cases} \tag{4}$$

If we have four pairs of (x, y) and (x', y'), we can compute the transform matrix H, thus to derive its inverse matrix H^{-1}. With the inverse matrix, we can compute the original image from the distorted one, thus to eliminate the projective distortion. In this paper, we used four internal corners we got to correct the image.

a b c

Fig.1 (a) Original image. (b) image with corners detected (c) image after correcting

Image segmentation. To get the region of interest is image segmentation. There are many methods to do segmentation. Some researchers used neural networks[9]. Although it can obtain good results, it needs a lot of training data, which will increase the amount of computation. In this paper, since the plants were green, we used the vegetation index[10] to extract the plants. The index is given as:

$$ExG - ExR = 2\mathrm{r} - g - b - (1.4r - g) \tag{5}$$

Where r, g and b are the normalized red, green, and blue chromatic coordinates of an image. They are given as:

$$\begin{cases} \mathrm{r} = \dfrac{\mathrm{R}}{\mathrm{R}+\mathrm{G}+\mathrm{B}} \\ \mathrm{g} = \dfrac{\mathrm{G}}{\mathrm{R}+\mathrm{G}+\mathrm{B}} \\ \mathrm{b} = \dfrac{\mathrm{B}}{\mathrm{R}+\mathrm{G}+\mathrm{B}} \end{cases} \tag{6}$$

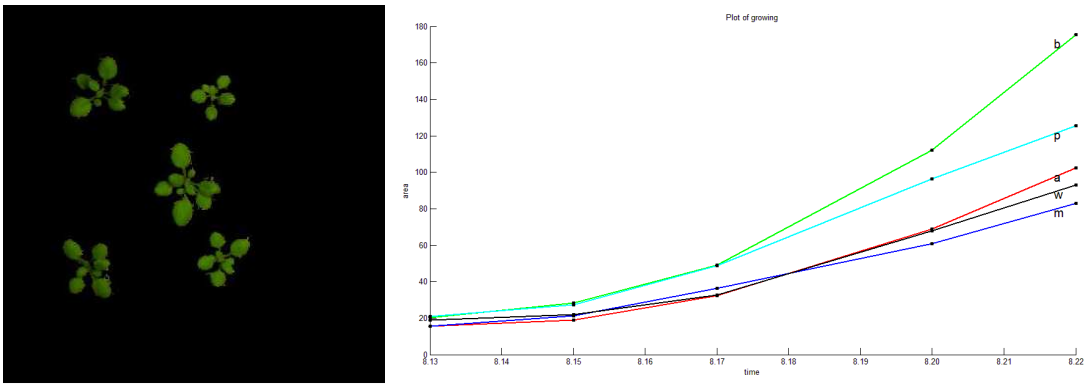
where R, G and B are the red red, green, and blue values of a pixel. For a pixel, if $ExG - ExR \geq 0$, then it belongs to green plants, otherwise, it is background point.

Area computation. After extracting the region of green plants, we could calculate the number of plants N_p. With the corner we got, we could also obtain the number of pixels of each grid of the checkerboard N_b in the image. Since we knew its real area S_b in advance, we could derive the area of one pixel, thus to compute the real area of the plants S_p. The equation is given as:

$$S_p = \frac{S_b}{N_b} \cdot N_p \tag{7}$$

Experiments

Fig.2(a) shows the segmentation result of one original image. With the result, we could calculate the average areas of the plants. Fig.2(b) illustrated the changes of leaf area of five kinds of Arabidopsis from 13 August to 22 August. This figure compares their growth rate in that period. From this figure, we can find that the over-expression types "b" and "p" grow faster than the other three types, while on contrary, the mutant "m", which without AMT1.1 gene, has the least growth rate after 17 August.



a b

Fig.2 (a) Image after performing segmentation (b) leaf areas of five kinds of Arabidopsis

Summary

This paper used image processing techniques to measure the Arabidopsis leaf area. The Harris corner detection algorithm was applied to find corners of the black and white checkerboard. The corners were used for correcting images, as well as to calculate the number of the grid pixels. The green plants were then segmented with the values of the red, green, blue components of each pixel. With the number of plant pixels in the image, as well as the number of checkerboard grid pixels and its real area, the real area of the plants were derived. This non-destructive method was used to conduct continuous measurement of Arabidopsis, thus to estimate the growth rate. It can also be applied to measure other green plants.

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