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# A NEW METHOD FOR MEASUREMENT OF ROOT LENGTH BY IMAGE PROCESSING

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CHIKUSHI J., YOSHIDA S. and EGUCHI H. *A new method for measurement of root length by image processing*. BIOTRONICS **19**, 129–135, 1990. Newly developed method of image processing was applied to measurement of the root length. The image of cucumber root system was taken by still video camera, and the root length was calculated on the basis of the counting operation of connected pixels in the thinned root images obtained from the image processor. Reliable estimations of thread length were provided with the designed counting technique. Estimation error was within 2% of actual length and the correlation coefficient between actual and estimated lengths was 0.9999 ($P<0.01$).

**Key words:** *Cucumis sativus* L.; cucumber; binary image; root length; image processing; thinning operation.

## INTRODUCTION

Characteristics of the plant root function closely relate to volume of the root system which can be estimated from total root length (*3*). There have been several techniques to measure or estimate root length such as the direct method, the inch counter method, and the line intersect method (*9*). In particular the line intersect method developed by Newman (*8*) has been widely used in the fields of environmental biology, plant physiology, soil science, and agronomy, etc. Furthermore, the method has been improved by using various instruments and devices (*1*, *2*, *4*, *6*, *7*, *10–14*).

The present paper deals with root length measurement by image processing in binary image operation, thinning operation, and counting of the connections of picture cells (pixels), and also deals with the application of the method to measurement of growth of the real root system in cucumber plant.

## MATERIALS AND METHODS

### *Material plant*

Cucumber plant (*Cucumis sativus* L. var. Hort. Chojitsu-Ochiai) were grown in a growth chamber at air temperature of 23°C, relative humidity of 70% and a light intensity of 200 $\mu$mol m$^{-2}$ s$^{-1}$ (12 h photoperiod) in hydroponic system. Detached root system was sampled at an interval of 3 days after one leaf stage.

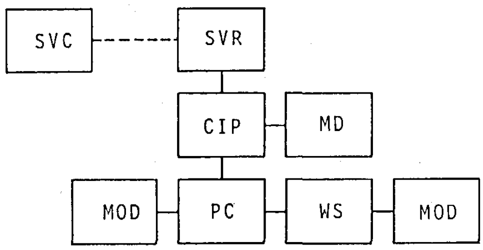


Fig. 1. Schematic description of the image processing system: SVC, still video camera; SVR, still video recorder; CIP, color image processor; PC, personal computer; MD, monitor display; WS, workstation; MOD, magneto-optical disk.

*Image processing*

A root sample was spread in water in order to eliminate the overlapped roots, where the root image was contrasted with black background. Figure 1 shows the image processing system. A still video camera (RC-474, Canon) including the charge-coupled device (CCD) was used for taking the images of the sample with a scale in a floppy disk. Image data were transmitted to the still video recorder (RR-551, Canon) through the floppy disk media. The root image was displayed on a CRT monitor (PC-TV454, NEC) with 3 modes (R, G and B) of 0 to 255 grades in brightness, and was operated to obtain the binary image through a graded filter (split filter) by using the image processor (Model ED-1382, Edec). The displayed image consisted of 512 lines$\times$480 pixels. Binary data of the pixels in one binary image was converted into ASCII data using personal computer (PC9801VX, NEC) for the thinning operation on the basis of the algorithm by Hilditch (*5*): The root image consisting of some pixels was changed to the lines passing through the centre of the root with one-pixel breadth. The thinning operation was conducted in a workstation (AS3160C, Toshiba) for the high-speed data processing. One-pixel length in the thinned image was evaluated from the interval of the scale marks on the display.

*Counting technique*

The total root length in one image can be estimated by using a thinned root image. When the thinned line consists of only vertical or horizontal connections of the pixels, the length of line can be evaluated by the number of pixels multiplied by one-pixel length. On the other hand, when the line declines with 45° to the horizontal, the length can be evaluated by 'the number of pixels$-$1' multiplied by $\sqrt{2}$ and one-pixel length, where $\sqrt{2}$ implies the correction factor for estimation length between two pixels connected diagonally. That is, a vertical or horizontal connection becomes shorter than a diagonal one even if the number of pixels is the same. So, we counted the total numbers of vertical or horizontal connections $H$ and diagonal connections $D$ respectively, because a root system was composed of various curved lines, i.e. diagonal and vertical or horizontal connections. Thus, total root length was evaluated by using these numbers in this experiment as follows,

$$R = (H+\alpha D)P \qquad (1)$$

Table 1. Effect of the correction of $\alpha$ on the measurement of thread length

| Actual length ($R$) (cm) | $\bar{\alpha}$ | Estimate (E1) by $\alpha=\sqrt{2}$ (cm) | $\lvert R-\mathrm{E1}\rvert/R$ (%) | Estimate (E2) by $\alpha=\bar{\alpha}$ (cm) | $\lvert R-\mathrm{E2}\rvert/R$ (%) |
|---|---|---|---|---|---|
| 40.2 | 1.273 | 42.15 | 4.85 | 40.02 | 0.45 |
| 49.4 | 1.314 | 51.48 | 4.21 | 49.13 | 0.75 |
| 63.9 | 1.293 | 66.92 | 4.72 | 63.85 | 0.07 |
| 92.2 | 1.271 | 95.85 | 3.95 | 91.12 | 1.17 |
| 100.7 | 1.308 | 104.99 | 4.26 | 100.52 | 0.18 |
| 151.6 | 1.257 | 156.81 | 3.43 | 149.16 | 1.61 |
| 199.7 | 1.280 | 209.94 | 5.12 | 199.33 | 0.19 |

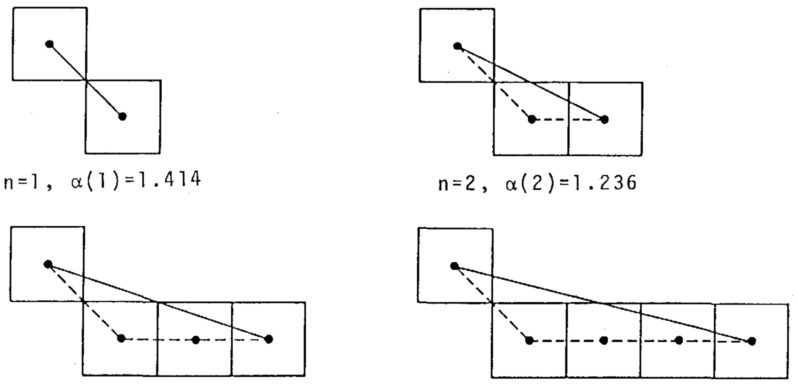


Fig. 2. Schematic diagram of pixel arrangements: $n$ and $\alpha(n)$ are numbers of the pixel and correction factor, respectively.

where $R$ is the total root length, $P$ is the one-pixel length and $\alpha$ is the correction factor. When $\alpha=\sqrt{2}$ was used for the evaluation of $R$, the total root length was overestimated by 3–5% of actual length (Table 1). To obtain more accurate estimation, $\alpha$ was corrected further. Figure 2 shows schematic diagrams for correcting of $\alpha$ in various cases of pixel arrangements. The gradient of the actual line of the root is variable, and various arrangements of the pixels were constituted according to degree of the gradient. This pixel arrangement was characterized by numbers $n$ of continuous pixels connected horizontally or vertically. In the case of $n\geqq 2$, the length shown in dashed line in Fig. 2 is measured to be longer than actual length of the root, and the assumed straight line (solid line) becomes necessarily closer to the actual length. Difference $d$ between these two lines is $(\sqrt{2}+1)-\sqrt{5}$. General $d$ in $n$ pixel connections can be calculated by

$$d(n)=(\sqrt{2}+(n-1))-\sqrt{(n^2+1)} \tag{2}$$

The integration of these differences caused distinct deviation from actual value when estimating the total root length. $\sqrt{2}$ in Eq. (2) corresponds to $\alpha$ in Eq. (1). When choosing $\alpha(n)$ value to become $d=0$, then general expression of $\alpha$ depending on $n$ is

$$\alpha(n) = \sqrt{(n^2+1)}-(n-1) \tag{3}$$

From the distribution of frequencies of connected pixels $M_n$, the weighted mean value $\bar{\alpha}$ of $\alpha(n)$ can be obtained by

$$\bar{\alpha} = \sum(\alpha(n)M_n)/\sum(M_n) \tag{4}$$

In this experiment, better estimations were obtained by using $\bar{\alpha}$ in stead of $\alpha=\sqrt{2}$ (Table 1). The estimation error was within 2% of the actual length.

## RESULTS AND DISCUSSION

Figure 3 shows original photograph (a), binary image (b) and thinned image (c) of the root system at 1 leaf stage of cucumber plant. The brightness of the root in the original image varied with the position and the diameter of the root. In the binary image, roots were contrasted clearly at even where the brightness was lower. In the thinned image, the tangled roots at the base of the root system were transformed into clearer lines than those in original or binary image. Although partial root image was lost by the thinning operation, it was negligibly small

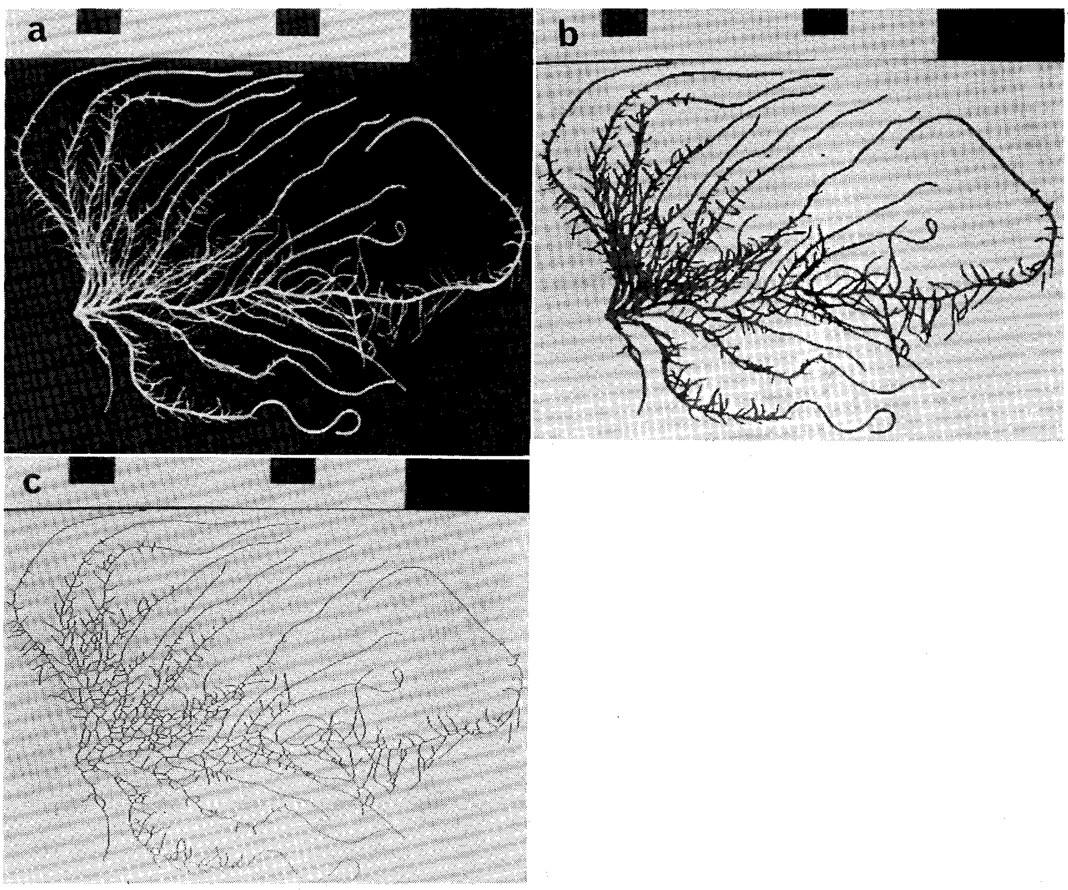


Fig. 3. CRT photographs of original image (a), binary image (b), and thinned image (c) of cucumber root system.

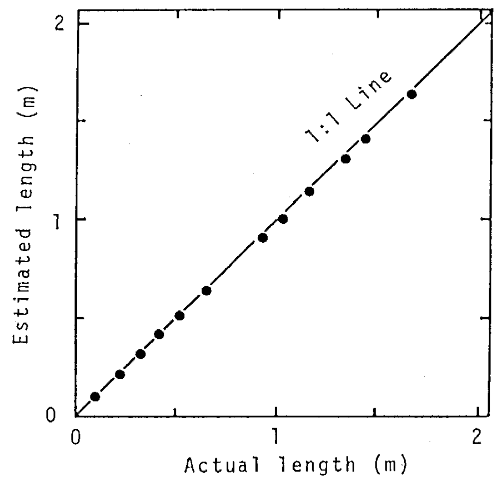


Fig. 4. Relationship between actual and estimated thread length.

Table 2. Accuracy of thread-length measurement by the present method

| Actual length (cm) | Estimated length (cm) | Standard deviation (cm) | Coefficient of variation (%) |
|---|---|---|---|
| 49.4 | 49.31 | 0.65 | 1.31 |
| 100.7 | 100.81 | 0.37 | 0.36 |
| 151.6 | 150.20 | 1.31 | 0.87 |
| 199.7 | 198.18 | 0.83 | 0.42 |

in total length of the root system.

The lengths of white threads were measured for the examination of accuracy in the present method. Figure 4 shows the relationship between actual and estimated values of thread lengths. Plotted marks almost distributed on the 1:1 line and correlation coefficient of the relationship was 0.9999 ($P<0.01$). Table 2 shows the comparison between actual and estimated thread lengths obtained from five samples for each length. The differences between these lengths were remarkably small. Furthermore, both the standard deviation and coefficients of variation were remarkably small. These facts suggest that a total root length can be estimated accurately and reproducibly.

The present method was applied to measurements of real root. Figure 5 shows growths of the root system and leaf expansion in cucumber plants. The root length in a root system was developed exponentially in course of time according to increases in root dry weight and leaf area per plant.

There were two advantages in the newly developed method for measuring root length; 1) randomness and direction of root images did not affect the measured root length, and 2) the image processing system available in commerce was able to be used for the measurement without any special instrument. Thus, the present method could be useful for the simple and accurate measurement of root length in various cases and may help to understand the characteristics of plant root system.

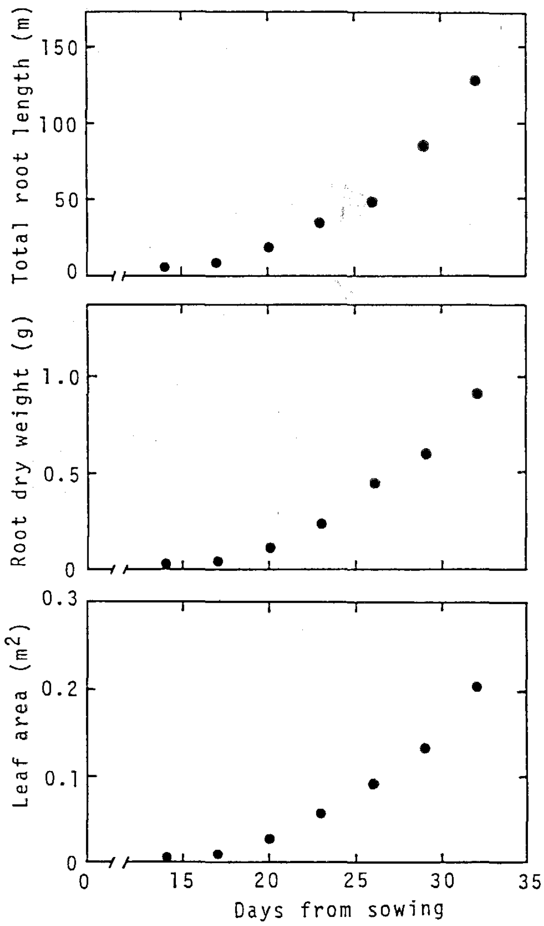


Fig. 5. Growths of root length, dry weight of root and leaf area in cucumber plants.

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