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Correlations between the Growth Period and Fresh Weight of Seed Sprouts and Pixel Counts of Leaf Area

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Abstract

Purpose: This study was carried out to predict the growth period and fresh weight of sprouts grown in a cultivator designed to grow sprouts under optimal conditions. **Methods:** The temperature, light intensity, and amount of irrigation were controlled, and images of seed sprouts were acquired to predict the days of growth and weight from pixel counts of leaf area. Broccoli, clover, and radish sprouts were selected, and each sprout was cultivated in a 90-mm-diameter Petri dish under the same cultivating conditions. An image of each sprout was taken every 24 hours from the 4th day, and the whole cultivating period was 6 days, including 3 days in the dark. Images were processed by histogram inspection, binary images, image erosion, image dilation, and the overlay image process. The RGB range and ratio of leaves were adjusted to calculate the pixel counts for leaf area. **Results:** The correlation coefficients between the pixel count of leaf area and the growth period of sprouts were 0.91, 0.98, and 0.97 for broccoli, clover, and radish, respectively. Further, the correlation coefficients between the pixel count of leaf area and fresh weight were 0.90 for broccoli, 0.87 for clover, and 0.95 for radish. **Conclusions:** On the basis of these results, we suggest that the simple image acquisition system and processing algorithm can feasibly estimate the growth period and fresh weight of seed sprouts.

Keywords: Fresh weight, Growth period, Image process, Leaf area, Seed sprout

Introduction

The consumption of seed sprouts, which has been ubiquitous for many centuries in oriental culture, has been increasing over the past 30 years (Robertson et al., 2002). The annual average growth of the Korean domestic vegetable industry increased by 0.5% and the value of sprouts and baby greens grew at an annual average of 23.9% from 2002 (7 million dollars) to 2006 (20 million dollars). Considering the potential demand for sprouts, the market is expected to grow to 100 million dollars by 2020. Furthermore, the amount of sprouts grown at home is increasing rapidly (Kim et al., 2006). This rise is because the consumption of sprouts is considered healthy since seed sprouts have more nutrients, such as vitamins, minerals, and phenolic compounds, than mature vegetables. These nutrients are 3–4 times greater in sprouts than in mature vegetables. Further, seed sprouts are low in calories, although they have numerous essential amino acids and unsaturated fatty acids (Jun et al., 2012).

Generally, the shelf life of fresh sprouts is very short, and the amount consumed at home is low. In addition, the cultivation of sprouts does not use pesticides, and therefore the mass production of sprouts leads to a high risk of infection by food pathogens such as *Escherichia coli* and *Salmonella* (Lee et al., 2009). To ensure the safety and freshness of sprouts, it is recommended that sprouts be

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grown at home, and it is wise to grow only a small amount of sprouts for one meal. Generally, seed sprout cultivation does not use pesticides because they might harm the sprouts' nutritional and biological characteristics, and sprouts are considered easier to grow at home than other vegetables. However, when growing sprouts at home, they can be exposed to an external environment that might have bacteria and harmful insects. When they are exposed, it is likely that sprouts will be contaminated by various viruses and toxic bacteria (Robertson et al., 2002).

There is limited previous research on household sprout cultivators. The existing commercial home sprout cultivator is well designed and can easily be used to grow various sprouts simultaneously. However, the optimal cultivation conditions for each type of sprout, such as the amount of water, humidity, temperature, and light, are not known (Pérez-Balibrea et al., 2008). Thus, it is necessary to design a home sprout cultivator that can control these cultivation conditions.

A study should be conducted to predict the fresh weight to determine the optimal harvest time. The nutritional benefits and chewiness of sprouts decline when the optimal harvest time is missed. Predicting the optimal harvest time saves energy and time and helps to produce uniform quality sprouts. The harvest time of sprouts can be decided by the fresh weight and the area of leaves, and these can be easily measured from an image of sprout leaves. Predicting the leaf area can easily be achieved using digital image processing. The image processing technique is a very convenient, rapid, and non-destructive method that does not affect the quality of objectives.

Mo et al. (2013) reported that image analysis based on the PLS-DA (partial least squares-discriminant analysis) could be used to sort high-quality seeds with a discrimination accuracy of over 99%. Additionally, Macfarlane et al. (2007) studied forest leaf area index estimates, which are important for modeling forest growth and water use. Sandmann et al. (2013) also conducted a study to predict the leaf area of kohlrabi and lettuce with an image processing method. The leaf area appears to be predicted with a high degree of accuracy, with the coefficient of determination being more than 0.97 and 0.99 for kohlrabi and lettuce, respectively. Studies of indicators that help to predict the leaf area are useful for determining the optimal harvest time and calculating the plant canopy. For instance, Bumgarner et al. (2012) have reported that significant correlations (r) of 0.85 to 0.96 ($P < 0.05$) were observed between measures of canopy cover calculated by image analysis and leaf area values obtained with a leaf area meter on lettuce. Furthermore, Tackenberg (2007) suggested a new method for the non-destructive measurement of biomass, growth rate, vertical biomass distribution, and dry matter content of diverse grass species based on digital image analysis. The developed linear regression models from the study showed no systematic errors compared with traditionally measured values and explained most of their variance ($R^2 > 0.85$ for all models).

The objective of this study is to develop an image processing algorithm for estimating the number of green pixels in an image and the fresh weight of popular sprouts such as broccoli, clover, and radish using a simple image acquisition system. The pixel value of green leaf area was analyzed and the pixel counts were used for the correlation analysis. The growth period and fresh weight were analyzed against the pixel counts in images to investigate whether pixel counts could determine the fresh weight and growth period of seed sprouts.

Materials and Methods

Cultivation conditions

In order to germinate and grow seed sprouts, appropriate light, temperature, humidity, and water irrigation amounts should be determined. The designed cultivator in this experiment was insulated by a 3 cm thickness of EPDM insulator and consisted of an embedded controlling board (FOX-D1004, DAE SUNG ENG., Korea), thermistor and humidity sensor (HIH-4030, Honeywell, USA), and irrigation controller (HRP-6M, HITECH, Korea). Because the air in the cultivator was heated or cooled by a Peltier element (TEC1-12715, Wellen, China) installed on the right side of the cultivator and was also heated by an LED near the top, this system contained two fans to circulate the air in the cultivator. Sprouts grown in the experiment included radish, clover, and broccoli, and low-quality seeds were selected because they are usually used for sprouts sold at the market to minimize the cost of cultivation.

One gram of each type of seed was spread on a 90-mm-diameter dish covered with two filter papers on the bottom to spread the irrigated water evenly. Generally, sprouts need a 3-day germination period that does not require light, and a 3-day growth period that requires light. Each dish was irrigated with 4 ml of water every 24

Table 1. Experimental sprout growth conditions

	Temperature (°C)	Humidity (%)	Light intensity	Irrigation
Germination period (Phase I, 3 days)	25–30	>90	0 μmol•m^{-2}•s^{-1}	Immersion
Growth period (Phase II, 3 days)	18–25	60–70	30 μmol•m^{-2}•s^{-1} (White)	4 ml every 24 hours

hours after sowing seeds. Sprouts were illuminated with white LEDs during the growth period. They were adjusted to an equal photosynthetic photon flux (PPF, 30 mol·m^{-2}·s^{-1}) using a quantum sensor (LI-COR, Lincoln, NE, USA)

In order to control the temperature, a Peltier element was installed on the right side of the cultivator. The surface temperature of a Peltier element can be controlled by switching the flow of the current and adjusting the current intensity. The air temperature inside the cultivator was maintained as uniform as possible so that the sprouts would be cultivated under the same conditions. Measuring the average temperatures from 4 thermistors located equidistant from each other and dividing the space into the same volume in the cultivator prevented any sprouts from receiving excessive heat. The specific growth conditions are presented in Table 1.

Image acquisition and data analysis method

Seventy of each type of sprout were cultivated. Twenty-one of each type of sprout were classified into three groups (4th, 5th, and 6th day) to clarify the relationship between the pixel count of leaf area and growth period. Further, the fresh weights of all sprouts were measured at different growth states to investigate the correlation between the fresh weight and the number of green pixels in images. The traditional correlation coefficient method was used to compare the similarity between fresh weights and pixel-count numbers in Microsoft Excel 2013 (Microsoft Corp., Redmond, WA, USA) using Equation 1.

$$r = \frac{\sum_{j=1}^{n}(x_j - \overline{x})(y_j - \overline{y})}{(n-1)\sigma_x \sigma_y} \qquad (1)$$

where r = the correlation coefficient between x and y
$\overline{x}$, $\overline{y}$ = the averages of x and y
σ_x, σ_y = the standard deviations of x and y
n = the number of samples

An RGB camera (A601fc Basler, Ahrensburg, Germany) was set up to acquire sprout images under consistent conditions as shown in Figure 1. The camera was installed at the top of a dark box and the sample was illuminated by 6 white LEDs. The dark box was hand built with dimensions of 250 mm × 400 mm × 900 mm (H).

In order to measure the pixel count of leaf area, images were analyzed to count the number of green pixels. The range of RGB was determined to determine the green area, and the ratios among R, G, and B values were considered to acquire the precise number of pixels of green area. The acquired images were processed using a binary image process in order to determine the pixels involved in the R, G, and B range. Next, an erosion process was applied to remove noise and outlying areas, and an image dilation process was applied to restore the leaf area removed by the erosion process. During the last step, an overlayer image process was applied to the original image to visualize the determined leaf area, and the pixels regarded as green area were counted. Matlab 2013b (Mathworks, Natick, MA, USA) was used to conduct all processes. The procedure of the image processing algorithm for calculating the pixel counts of sprout green area is shown in Figure 2.

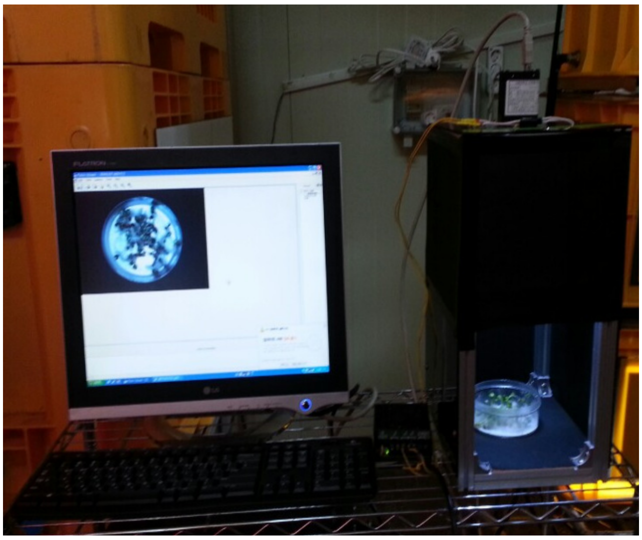

Figure 1. Acquisition of a sprout image using the image acquisition system.

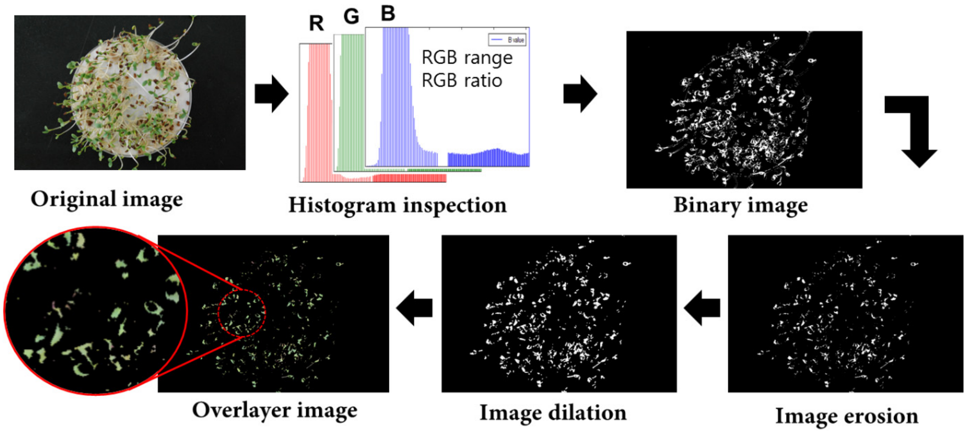

Figure 2. Procedure of image processing to calculate the pixel counts of leaf area.

Table 2. Ranges of R, G, and B values of each sprout used to count pixels for leaf area. M-2σ is the minimum and M+2σ is the maximum value in the range. Negative values were considered to be zero

	R			G			B		
	Mean	M-2σ	M+2σ	Mean	M-2σ	M+2σ	Mean	M-2σ	M+2σ
Broccoli	112.13	24.52	199.74	163.83	86.23	241.43	65.16	0	135
Clover	117.69	28.23	207.15	167.07	96.83	237.31	130.91	59.16	202.66
Radish	126.35	69.03	183.67	166.04	108.36	223.72	42.66	1.41	83.91

Results and Discussion

Images were taken starting on the 4th day of growth when green leaves appeared. The number of green pixels was calculated from the acquired images. By investigating 200 pixels of leaves for each species, the range of RGB values was determined according to the mean ± 2σ as shown in Table 2.

The leaf area processed by the image processing algorithm using the determined range of RGB values of each sprout is shown in Figure 3. In order to solve the problem of unexpected areas such as leaf shadows or light refection on water, some pixels were eliminated by setting the ratio boundary between R or B and G. The boundary was determined by the average ratio of 200 pixels extracted from images of each species. Although some noise pixels were eliminated by the image erosion and image dilation process, some were still left. However, these could be ignored because they were very few compared to the total pixel counts of leaves.

The calculated pixel counts in images from the 4th, 5th, and 6th days are shown in Figure 4. The pixel counts and the growth period were closely related for broccoli, clover, and radish. The coefficients of correlation were 0.91 (broccoli), 0.98 (clover), and 0.97 (radish). Images of each

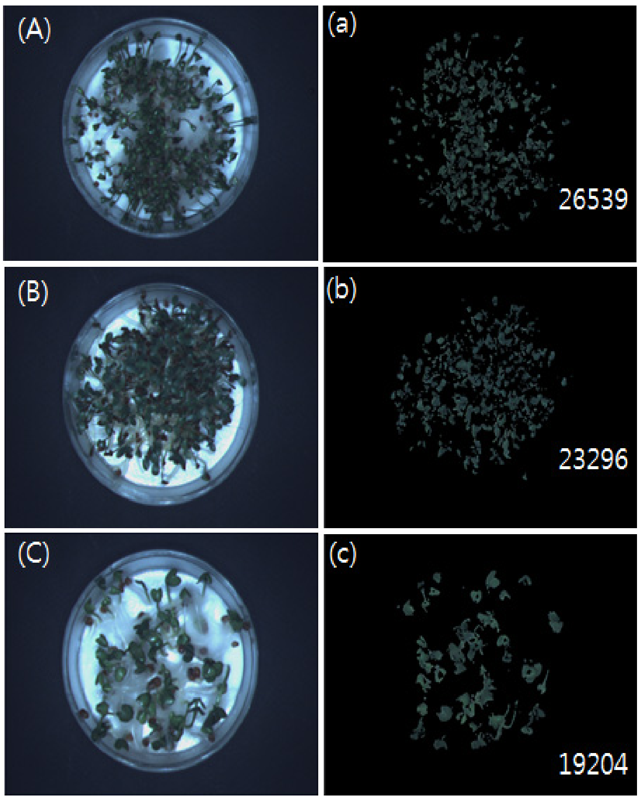

Figure 3. The original image (left) and the processed image showing only the green area of each sprout (right). The numbers on the figure indicate the total green area pixel count; (A)-(a) broccoli, (B)-(b) clover, (C)-(c) radish.

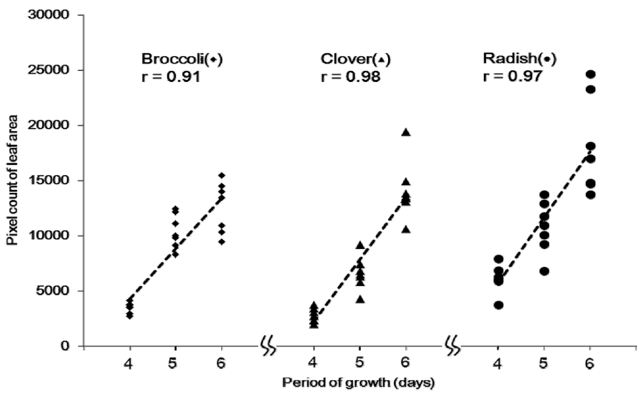

Figure 4. Pixel count measured by the image processing algorithm for each sprout on the 4th, 5th, and 6th days. Coefficients of correlation are presented on the graph.

crop from the 4th and 5th days of cultivation could be separated by a pixel count of 5000. Images from the 5th and 6th day could be separated by pixel counts of 10,000 and 15,000 for clover and radish, respectively. These results showed that the relationship between the pixel count of leaf area analyzed by the image process and the growth period were highly correlated. Therefore, it should be possible to predict the fresh weight and growth period of seed sprouts using image analysis.

The fresh weight of sprouts in each dish was measured using a digital scale (Explorer Pro, Ohaus Corporation, Pine Brook, NJ) after removing moisture in order to investigate the relationship between the pixel count of leaf area and fresh weights. The relationship between the fresh weights and the calculated pixel counts in images is shown in Figure 5. The relationships between the pixel count and fresh weight for broccoli, clover, and radish were highly correlated, with correlation coefficients of 0.90, 0.87, and 0.95, respectively.

Clover had higher pixel counts than broccoli or radish. This was because clover had more seeds per unit weight than the other species. The greater number of seeds for clover meant that there were more leaves per dish; therefore, leaves were more likely to overlap, leading to the lower correlation coefficient for clover. Further, it was determined that the greater leaf area from the same weight of seed was because clover puts more resources into the growth of leaves than into roots and stems.

Conclusions

In this study, the pixel count of leaf area and the growth period were highly correlated, with correlation coefficients of 0.91, 0.98, and 0.97 for broccoli, clover, and radish, respectively. These results suggest that the growth rate could be predicted by the pixel count of leaf area analyzed by image processing. Furthermore, the correlation coefficients between the pixel count of leaf area and the fresh weight were 0.90 for broccoli, 0.87 for clover, and 0.95 for radish. On the basis of these results, we suggest that the simple image acquisition system and processing algorithm could feasibly estimate the growth period and fresh weight of seed sprouts.

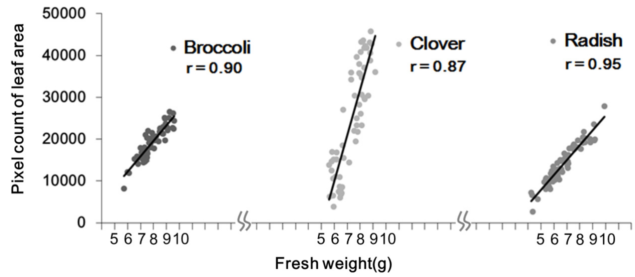

Figure 5. Correlation coefficient between the pixel counts measured by the image processing algorithm and fresh weights.

Conflict of Interest

The authors have no conflicting financial or other interests.

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