Kinetic Measuring Method of Rice Growth in Tillering Stage Using Automatic Digital Imaging System

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We developed an automatic digital imaging system to acquire sequential growth images of rice in tillering stage. In this system, we cultivated 24 plants and acquired images of each individual plant at one-hour intervals. We measured leaf height using this system for 36 days from these sequential images. These sequential leaf height measurements made it possible to quantify the precise growth of rice plants, showing the timing of leaf elongation, leaf-emergence interval and leaf elongation rate. We applied this method to evaluate the effect of photoperiod and temperature on rice growth. The timing of leaf emergence and the leaf-emergence interval were affected by temperature and day length. On the other hand, the maximum leaf elongation rate was not significantly different under the temperature and day length examined. These results suggest that temperature and day length have different effects on leaf emergence and leaf elongation in rice. This method and instrument could be applied to phenotypic profiling studies for comprehensive growth of various plant species and cultivars.

Keywords: image, leaf elongation rate, leaf-emergence interval, leaf growth, rice

INTRODUCTION

As rice genome sequencing is nearly complete (Sasaki et al., 2002), a systematic and comprehensive measurement of whole-plant phenotypes is desirable. To determine the functions of genes, differences of visible phenotypes have been found after comparing mutants and transgenic plants with wild types. Visible phenotypes have been observed in mature rice plants, including plant shape, plant height, and heading day (Takano et al., 2001). Such visible phenotypes are related to either the mutated genes or the transformed genes. Comprehensive measurement of visible phenotype is especially important because it cannot be predicted when and where changes of the plant form emerge in the growth process. The number of mutants and transgenic plants for gene functional analysis is increasing due to progress in the techniques for producing them (Hirochika et al. 1996). Therefore, it is indispensable to measure many plants that have different genetic backgrounds grown under identical conditions.

Plant growth is affected by various environmental elements including temperature, photoperiod, nitrogen supply and water temperature (Amin et al., 2002; Sasaki, 2002; Goto, 2003). The effect of temperature and day length on plant growth has been previously studied by using the plant shape of various plants. Thomas and Vince-Prue (1997) reported that the day length affect
leaf expansion of plants; they found that individual leaves were larger in long-day conditions than in short-day conditions in many plant species.

In conventional methods of analyzing the whole-plant phenotypes in rice, plant forms were measured with a ruler or just by counting the number of leaves and tillers. Katayama, in 1951, marked and recorded the day of leaf emergence to investigate the correlation of leaf growth in a main culm and tillers for about one hundred days. That method was effective for determining the entire number of leaves and tillers in a plant as well as for measuring the actual length of leaf blades at a certain time. Precise measurement of leaf elongation and area expansion, however, was practically impossible at intervals of less than 24 h. It was also difficult to accurately measure the timing of leaf emergence.

To solve these problems, a computational method has been developed to measure the growth of horizontal projected leaf area (Shibata et al., 1993) and the velocity of areal growth in a plant leaf (Schmidt and Schurr, 1999). An automated measuring system using five cameras simultaneously was also developed to record rhythmic motions of some plant organs (Yamashita and Miwa, 1995). An automated video imaging system showed that hypocotyl elongation in Arabidopsis thaliana was controlled by circadian clock at the early stages of seedling development (Dowson-Day and Millar, 1999). This system controlled up to 14 cameras and captured images of up to eight seedlings per camera. In these methods, it was difficult to acquire images of dozens of middle seedlings and mature plants simultaneously because both the cameras and plants were in a fixed position.

In this study, we developed an automatic digital imaging system composed of a camera system and a plant conveying system to acquire growth images of 24 rice plants at one-hour intervals for 36 days. We measured leaf height from these sequential images using newly developed software. The measurement of leaf height made it possible to quantify the growth of rice plants, showing the timing of leaf emergence, leaf-emergence interval and leaf elongation rate. We applied this method to evaluate the effect of photoperiod and temperature on rice growth. Finally, we discussed a future phenotypic profiling method for comprehensive plant growth.

MATERIALS AND METHODS

1. Imaging system

We designed and built an automatic digital imaging system to acquire sequential images of rice growth in tillering stage. The scheme of the imaging system is shown in Fig. 1A. We call this system the “Hitachi Rice Imaging System type Junior” (HI-RIS Junior) in the present study. It acquires sequential images of the growth process of each individual seedling at regular intervals by a control program. We cultivated 24 plants in flowerpots (1/5,000 are Wagner pot (158 mm in diameter), Fujimotokagaku, Tokyo) (Fig. 1B) placed in a growth chamber (width 2,500 mm, depth 2,100 mm, height 1,800 mm; Koitotoron KG-V65 special type, Koito Ind., Tokyo). Twelve flowerpots were connected into a unit, and two units were set up in elliptical orbits at equal intervals (Fig. 1B).

Growth images were captured by the following procedure. According to the programmed time interval, flowerpots moved at a speed of 69 mm s⁻¹ along the orbit, and a plant was conveyed into the imaging position in front of the camera (Fig. 1B). The plant at the imaging position was raised by 450 mm to take whole plant images without being hindered by the neighboring plants (Fig. 1A). After imaging was finished, the plant was returned to the orbit and moved forward one position. The same operation to capture images was repeated twelve times per circuit. It takes 28 min to make around on the circuit. Images were acquired in the light period, but not in the dark period. The accuracy of the position was measured by coordinates of a position marker placed on a flowerpot. The variance of the coordinates of the marker position was less than three pixels (which
KINETIC MEASURING METHOD USING AUTOMATIC DIGITAL IMAGING SYSTEM

Fig. 1 Automatic digital imaging system to acquire growth images of rice. (A) Photograph of the system: A plant conveyed to an imaging position is raised up to acquire images without hindrance from neighboring plants. (B) Arrangement of plants and cameras. (C) Two types of cameras in HI-RIS Junior. Camera 1: the fixed camera for full-length images. Camera 2: the movable camera for detailed observation.

corresponded to 1.0 mm) either horizontally or perpendicularly.

HI-RIS Junior has two imaging mechanisms to observe a plant from multiple viewpoints. First, a revolving stage rotates plants at ten degrees per second and images are captured from multiple angles (Fig. 1A). This is effective in obtaining growth information by ensuring three-dimensional arrangements of each organ. Second, two kinds of cameras are used for imaging plants from different distances (Fig. 1B). Camera 1 (color digital camera DFW-X700, SONY; C-mount lens JF4.8 1.8, Canon) acquired full-length images (Fig. 1C). Camera 2 (same camera; C-mount lens PHF6 1.4, Canon) acquired magnified images of specific figures to observe in detail (Fig. 1C). The images acquired by HI-RIS Junior were conveyed one by one to computer directories prepared for each rotation angle.

Figure 2 shows sequential growth images of Norin 8 that were cultivated under long-day conditions (LD: 14 h light / 10 h dark) at 30°C light / 25°C dark. Images were taken by camera 2 from
Fig. 2 Sequential growth images of Norin 8. Images show the growth of Norin 8 cultivated under long-day conditions (14 h light/10 h dark) at 30°C light/25°C dark from 12:00 on the 21st day to 18:00 on the 23rd day after sowing. The images were captured at one-hour intervals by camera 2.

12:00 on the 21st day to 18:00 on the 23rd day after sowing (see Fig. 1B). We observed these sequential images as a short movie and found that we could recognize the growth process of each leaf from emergence to the end of elongation.

2. Plant material and cultivating condition


Seeds were surface-sterilized and sown on 0.2% gellan gum containing half concentration of Murashige and Skoog plant salt mixture (MS, Murashige and Skoog, 1962) in test tubes. The seeds were incubated and cultivated under 12 h light / 12 h dark conditions at 28°C in a growth chamber (Koitotoron, FR-6113N, Koito, Tokyo) for 7 days. Then we transplanted the seedlings into soil (Bonsol-2, Sumitomo Chemical, Osaka) in flowerpots. They were cultivated for 50 days in HI-RIS Junior under LD at 28°C light / 23°C dark, and 75% humidity, unless otherwise indicated. When we defined the effect of light and temperature conditions on the rice growth, we cultivated Norin 8 under short-day conditions (SD: 10 h light / 14 h dark) and LD, in combination with two different temperature controls (light 28°C / dark 23°C and light 30°C / dark 25°C). The photosynthetic photon flux (PPF) at the distance of 1,000 mm from light sources in the chamber of HI-RIS Junior was
KINETIC MEASURING METHOD USING AUTOMATIC DIGITAL IMAGING SYSTEM

Fig. 3 Measurement of leaf height. (A) Definition of leaf height: leaf height was measured as the distance from the ground level to the highest point of each leaf on the image. \( y_0 \) corresponds to the \( y \) axis component of the coordinates of the ground position, and \( y_n \) corresponds to that of the highest point on the \( n \)th leaf. (B) The flow for measuring leaf height.

530 \( \mu \text{mol m}^{-2} \text{s}^{-1} \). We did not add any fertilizer during the cultivating period. Four plants were cultivated and their leaf heights were measured under the same environmental conditions.

3. Measurement of leaf growth using sequential images

Plant height used in plant biology is defined as the vertical distance from the ground to the highest point of a plant (Fig. 3A). Similarly, we defined leaf height as the distance from the ground to the highest point of each leaf using sequential images of HI-RIS Junior (Fig. 3A). We measured leaf height individually in 500 sequential images captured at one-hour intervals by camera 1 (Fig. 1B and 1C) for 36 days. To take this measurement quickly, we developed a software program called “HI-RIS viewer”. Using HI-RIS viewer, we observed images sequentially and recorded the coordinates of the measuring points in pixel units. The highest point of each leaf was designated manually by a mouse operation, and the data were saved automatically as a comma separated values (CSV) file (Fig. 3B).

Calculation of leaf height in millimeter units was performed by the following equation:

\[
\text{Leaf height (mm)} = |y_0 - y_n| \times C \text{ (mm pixel}^{-1}\text{)}
\]

where \( y_0 \) corresponds to the \( y \) axis component of the coordinates of the ground point, and \( y_n \) corresponds to that of the highest point of the \( n \)th leaf (Fig. 3A). \( C \) is the coefficient of a pixel on the images. In this study, \( C \) was 1.03 (mm pixel\(^{-1}\)) in camera 1.

RESULTS

1. Accuracy of leaf height on images

The accuracy of leaf height on images in comparison to the actual leaf height was examined. It depends on the three-dimensional angle of a leaf, including its angle \( \alpha \) of inclination against perpendicular and its rotation angle \( \theta \) from the camera position (Fig. 4A). We measured leaf height on the images using straight leaves connected with a scale of 600 mm in length at various inclination angles \( \alpha \) (0°-15°) and rotation angles \( \theta \) (0°-360°) (Fig. 4B). At the inclination angle \( \alpha \) of 0°, both actual leaf height and leaf height on image were 600 mm (Fig. 4B). When the leaf inclination
Fig. 4 Range of calculated leaf height on images. (A) Leaf inclination angle ($\alpha$) indicates the angle at which a leaf is inclined from the vertical. Plant rotation angle ($\theta$) indicates the angle between a leaf and a camera. (B) Leaf height was measured on image when the leaf inclines to 0, 5, 15° from the vertical. The rotation angle is $0^\circ$ when a leaf inclines towards a camera.

angle $\alpha$ was 5°, the actual leaf height was 598 mm and the leaf height on image was calculated as 580–610 mm (Fig. 4B). Furthermore when the leaf inclination angle $\alpha$ was 15°, the actual leaf height was 580 mm and the leaf height on image was calculated as 548–610 mm (Fig. 4B). The range of calculated leaf height on images increased as the inclination angle $\alpha$ increased, because of the increase of the range of distance between the camera and the leaf apex.

We measured actual angles of inclination of the main culm and primary tillers 1 and 2 on the 25th day after sowing (Table 1). The leaf inclination was equivalent to the inclination of main culm and tillers themselves, because the leaves grew almost straight to either the main culm or tillers during their early growth. The inclination angle of the main culm was $1.3\pm0.8^\circ$. The leaves of the main culm grew almost vertically, therefore, the leaf height measured on images was constant irrespective of the leaf rotating position (Fig. 4B). The leaf height of tillers measured on im-

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Table 1 Actual angles of inclination of main culm, primary tillers 1 and 2 of Norin 8 on the 25th day after sowing. Angles were measured by protractor.

<table>
<thead>
<tr>
<th></th>
<th>Angle (degrees)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main culm</td>
<td>1.3±0.8</td>
</tr>
<tr>
<td>Primary tiller 1</td>
<td>16.3±2.5</td>
</tr>
<tr>
<td>Primary tiller 2</td>
<td>14.4±1.0</td>
</tr>
</tbody>
</table>

Values are means ± standard errors ($n=8$).
ages should have some range less than 5.6% of the actual leaf height depending on the plant rotating position (Table 1, Fig. 4B).

2. Detection of growth parameters from sequential leaf height data

To precisely quantify the growth of rice plants in tillering stage using the sequential leaf height data, we defined growth parameters including timing of leaf emergence, leaf-emergence interval, maximum leaf elongation rate, leaf emergence position, maximum leaf height and leaf elongating duration. In this study, we focused on the timing of leaf emergence, the leaf-emergence interval and the maximum leaf elongation rate to characterize leaf growth as a function of time. Leaf emergence was defined as the time when the tip of a new leaf blade protruded from the main culm and reached the length of 20 mm. The timing of leaf emergence indicated the duration from sowing to each leaf emergence. The leaf-emergence interval (LEI) was estimated from the time one leaf emerged until the following leaf emerged. LEI-\(n\) was defined as the time interval between the time when the (\(n\))th leaf emerged and the time when the (\(n+1\))th leaf emerged (Fig. 5). We defined maximum leaf elongation rate (MLER) as the average elongation rate of the first two days after leaf emergence. MLER-\(n\) was defined as the maximum elongation rate of the (\(n\))th leaf. Figure 5 shows the sequential leaf height data of the eighth and the ninth leaves in the growth of Nipponbare.

To prove the usefulness of sequential leaf height data for the detection of plant growth, we compared the leaf height data of Nipponbare in tillering stage with the plant height data measured by the conventional method and by HI-RIS Junior. Figure 6A shows sequential measurements of plant height using the conventional method. The highest leaf in a plant was selected and its height was measured by a ruler once a week (Fig. 6A). The conventional method showed the plant height elongated at a fairly regular speed of 17 mm day\(^{-1}\). We also measured plant height once an hour during day time from sequential images acquired by HI-RIS Junior (Fig. 6B). The growth curves illustrated the phased increase in plant height, including repetitive elongation and stagnation of leaves (Fig. 6B).

In contrast, we measured each leaf height of the main culm of a plant using sequential images acquired by HI-RIS Junior (Fig. 6C). Figure 6C indicates the subsequent emergence and elongation of new leaves in a plant. We proved that new leaves emerged periodically in the main culm.
Fig. 6 Time course of plant height and leaf height. Nipponbare was cultivated under long-day conditions (14 h light/10 h dark) at 28°C light/23°C dark. (A) Plant height was measured by a ruler once a week. (B) Plant height was measured by HI-RIS Junior at one-hour intervals. (C) Leaf height was measured by HI-RIS Junior from the fifth to the eleventh leaves in the main culm.

(Fig. 6C). They emerged within an average interval of $3.9 \pm 0.2$ days in the fifth to the eleventh leaves of a main culm under our experimental condition. The maximum inclinations of leaf growth curves were very similar within a plant (Fig. 6C). The average value of MLER of each leaf was $3.4 \pm 0.3$ mm h$^{-1}$.

We found that the growth curves shown in Fig. 6B reflected the later stage of growth of individual leaves (see Fig. 6C). The elongation rate of each leaf height determined from Fig. 6C was five times greater than the average elongation rate of plant height determined from Fig. 6A.

3. Synchronicity of leaf growth in main culm and tillers

We examined quantitatively the relation of leaf growth between the main culm and tillers in a plant. Using our system, we separately identified the third to the eleventh leaves of a main culm, the first to the sixth leaves of primary tillers 1 and 2, and the first to the third leaves of primary tiller 3 on sequential images. We were unable to identify the leaves emerging thereafter, because of hindrance by other leaves and tillers.

Figure 7A shows that the images of Norin 8 cultivated under LD at 30°C light / 25°C dark on the 22nd day after sowing. When the eighth leaf in the main culm emerged, both the third leaf in primary tiller 1 and the second leaf in primary tiller 2 emerged simultaneously. Then, they elongated at a nearly equal growth rate $(4.7 \pm 1.3$ mm h$^{-1}$) for 10 h.

Using sequential images, we measured leaf height of the eighth to the eleventh leaves in the main culm, the third to the sixth leaves in primary tiller 1 and the second to the fifth leaves in primary tiller 2. Figure 7B shows representative data of Norin 8 that was cultivated under LD at 28°C
KINETIC MEASURING METHOD USING AUTOMATIC DIGITAL IMAGING SYSTEM

Fig. 7 Time course of leaf length in the main culm and tillers. (A) The left photograph is the image of Norin 8 cultivated under long-day conditions (14 h light/10 h dark) at 30°C light/25°C dark acquired at 8:00 on the 22nd day after sowing, and the right photograph is that at 18:00 on the same day. The third leaf in primary tiller 1 (b) and the second leaf in primary tiller 2 (c) emerged and elongated with the eighth leaf in a main culm (a) simultaneously. (B) The graphs show the leaf length measurements of the 8th to the 11th leaves in a main culm, the 3rd to the 6th leaves in primary tiller 1, and the 2nd the 5th leaves in primary tiller 2 under long-day conditions (14 h light/10 h dark) at 28°C light/23°C dark. Open and closed arrows indicate synchronous emergence and elongation, respectively.

light / 23°C dark. Synchronous leaf emergence and synchronous leaf elongation were demonstrated, especially among the tenth leaf in the main culm, the fifth leaf in primary tiller 1 and the fourth leaf in primary tiller 2, as well as among the eleventh leaf in the main culm, the sixth leaf in primary tiller 1 and the fifth leaf in primary tiller 2 (Fig. 7B).

4. Effect of day length and temperature on rice growth parameters

To define the effect of day length and temperature conditions on the rice growth parameters, we cultivated Norin 8 under four different environmental conditions and compared growth parameters including timing of leaf emergence, LEI and MLER.

Figure 8 shows the representative time course of their leaf height growth. The timing of leaf emergence (Table 2), LEI (Table 3) and MLER (Table 4) were determined from Fig. 8 and the other data of plants cultivated under the same growth conditions.

Table 2 demonstrates the timing of leaf emergence from the seventh leaf to the tenth leaf.
Under both LD and SD, each leaf emergence under 30°C/25°C conditions was earlier than those under 28°C/23°C conditions. When plants were grown under 28°C/23°C conditions, each leaf emergence was earlier for plants grown under LD than those under SD. Under 30°C/25°C conditions, the emergence of the seventh leaf grown under LD was almost the same with that grown under SD.

Table 3 shows LEI between the seventh and the eighth leaves (LEI-7) and between the ninth and the tenth leaves (LEI-9). In each environmental condition, LEI-9 was longer than LEI-7.

Table 2  The timing of leaf emergence. The 7th leaf to the 10th leaf in the main culm of Norin 8 were observed.

<table>
<thead>
<tr>
<th>Growth condition</th>
<th>7th leaf</th>
<th>8th leaf</th>
<th>9th leaf</th>
<th>10th leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>28°C/23°C</td>
<td>20.5±0.3 a*</td>
<td>23.7±0.1 a*</td>
<td>27.6±0.2 a*</td>
<td>31.6±0.5 a*</td>
</tr>
<tr>
<td>28°C/23°C</td>
<td>22.3±0.3 b*</td>
<td>27.0±0.4 b*</td>
<td>31.4±0.3 b*</td>
<td>36.4±0.4 b*</td>
</tr>
<tr>
<td>30°C/25°C</td>
<td>18.9±0.5 c*</td>
<td>21.9±0.4 c*</td>
<td>24.7±0.5 c*</td>
<td>28.6±0.5 c*</td>
</tr>
<tr>
<td>30°C/25°C</td>
<td>19.3±0.7 c*</td>
<td>22.4±0.9 c*</td>
<td>25.9±1.0 c*</td>
<td>30.1±1.3 c*</td>
</tr>
</tbody>
</table>

Values are means± standard errors with results of statistical analysis (n = 4).
* Means in each column followed by the same letters are not significantly different at P < 0.05 level by t tests.
KINETIC MEASURING METHOD USING AUTOMATIC DIGITAL IMAGING SYSTEM

Table 3  The leaf-emergence interval in main culm of Norin 8. LEI-n means the time interval between the emergence of the (n)th leaf and that of the (n + 1)th leaf.

<table>
<thead>
<tr>
<th>Growth condition</th>
<th>Leaf-emergence interval (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LEI-7</td>
</tr>
<tr>
<td></td>
<td>LEI-9</td>
</tr>
<tr>
<td>28°C/23°C</td>
<td>LD 3.2±0.3 a*</td>
</tr>
<tr>
<td></td>
<td>SD 4.7±0.3 a*</td>
</tr>
<tr>
<td>30°C/25°C</td>
<td>LD 3.0±0.3 b*</td>
</tr>
<tr>
<td></td>
<td>SD 3.1±0.3 b*</td>
</tr>
</tbody>
</table>

Values are means ± standard errors with results of statistical analysis (n=4).
* Means in each column followed by the same letters are not significantly different at P<0.05 level by t tests.

Table 4  Maximum leaf elongation rate from the 7th leaf to the 10th leaf in the main culm of Norin 8. The maximum leaf elongation rate was defined as the average elongation rate of the first two days from leaf emergence timing. MLER-n was defined as the maximum elongation rate of the (n)th leaf.

<table>
<thead>
<tr>
<th>Growth condition</th>
<th>Maximum leaf elongation rate (mm h⁻¹)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MLER-7</td>
</tr>
<tr>
<td></td>
<td>MLER-8</td>
</tr>
<tr>
<td></td>
<td>MLER-9</td>
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<td></td>
<td>MLER-10</td>
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<tr>
<td>28°C/23°C</td>
<td>LD 3.5±0.3 a*</td>
</tr>
<tr>
<td></td>
<td>SD 3.2±0.2 a*</td>
</tr>
<tr>
<td>30°C/25°C</td>
<td>LD 4.5±0.4 b*</td>
</tr>
<tr>
<td></td>
<td>SD 3.9±0.2 a*</td>
</tr>
</tbody>
</table>

Values are means ± standard errors with results of statistical analysis (n=4).
* Means in each column followed by the same letters are not significantly different at P<0.05 level by t tests.

When we compared the LEI under four different environmental conditions, LEI under SD in 28°C/23°C conditions was longer than that under other conditions.

Table 4 shows MLER from the seventh leaf (MLER-7) to the tenth leaf (MLER-10). MLER was not significantly different between the environmental conditions examined.

As a result, the timing of leaf emergence and LEI were affected by temperature and day length. LEI was 36% shorter (1.7 days shorter) under LD in 30°C/25°C conditions than under SD in 28°C/23°C conditions. On the other hand, the leaf elongation can be almost constant regardless of temperature and day length.

DISCUSSION

1. Usefulness of growth parameter measured by newly developed imaging system for detection of plant growth

In this study, we designed an automatic digital imaging system that acquired sequential images of whole plants at short time intervals through tillering stages, to comprehensively analyze the time progression of visible phenotypes.

Systems that acquire images of whole plants have been previously developed. Shibata et al. (1993) developed a system for measuring the horizontal projected leaf area of lettuce plants. Images of plants were acquired for thirty days after sowing at intervals of several days. The data of the horizontal projected leaf area obtained from images made it possible to estimate the top fresh weight. A similar system was developed to detect the cabbage head and to measure head size using images (Hayashi and Sakaue, 1996). The measured diameter from images of the horizontal leaf area gave an appropriate index to judge harvesting time for cabbage heads.

Other research groups investigated imaging systems to detect the rhythmic motion or velocity...
of plant growth. Yamashita and Miwa (1995) developed a system to record rhythmic motion of a plant every minute using image processing technology. They observed that roots and leaves of a pea had a rhythmic motion of one- or two-hour intervals. Another system was developed to measure the velocity of Ricinus leaf growth (Schmundt and Schurr, 1999). Van der Weele et al. (2003) developed a microscopic imaging system to measure the velocity of root tip growth using sequential images taken every five or ten seconds. The algorithms based on image sequence analysis do not need tedious manual measurements. Those previous results proved that the imaging systems made it possible to detect movement and growth rate of plant that were not detected by the conventional method.

We proved that HI-RIS Junior was useful to detect plant growth, especially of rice. Our system measured in detail the elongation of plant height as whole plant growth through tillering stage (Fig. 6B). We measured the elongation of each leaf height at the same time when we measured plant height (Fig. 6C). Furthermore, we detected not only synchronous leaf emergence but also synchronous leaf elongation in the main culm and the tillers (Fig. 7). Using the imaging system, we were able to recognize simple regularities existing in rice growth dynamics.

Synchronous emergence of leaves in the main culm and tillers in rice plants has been reported by Katayama (1951). He observed and marked leaves of Norin-6 grown in flowerpots over approximately one hundred days, placing marks on newly spread leaf blades each day. That method specified the emergence timing of each leaf, however, the elongation rate was not measured. Leaf growth rate was measured in a separate experiment limited to leaves of a main culm (Katayama, 1942). We showed that our imaging system made it possible to measure both leaf emergence and leaf elongation rate simultaneously.

Our results suggest that kinetic measurement of growth using the imaging system would be useful in analyzing detailed phenotype of mutants and transgenic plants in the future.

2. Effect of environmental condition on leaf growth of rice

The effect of temperature and day length on plant growth has been studied in various plants. Huang et al. (2001) reported that the leaf emergence and the shoot elongation were affected by temperature in common lambsquarters. Tamaki et al. (2002a) reported that both leaf emergence rate and leaf area growth rate increased with increasing temperature until reaching to an optimum temperature in barley plants. Similarly, both leaf emergence rate and leaf area growth rate increased with the increase of day length (Tamaki et al., 2002b).

In the present study, we measured LEI and MLER in the growth of rice plants. LEI under the SD conditions was shorter under the high temperature conditions than under the low temperature conditions (Table 3). LEI under the low temperature conditions was shorter under the LD conditions than under the SD conditions (Table 3). Thus, LEI was affected by both temperature and day length. On the other hand, MLER was not significantly different under the temperature and day length conditions examined (Table 4).

In rice, the leaf-emergence interval and the leaf elongation rate had been considered to have a correlation (Hoshikawa, 1989). Here we found that MLER did not always correlate with LEI in a precise growth measurement using HI-RIS Junior. When rice plants were grown under LD in 30°C/25°C conditions, MLER of the seventh to ninth leaves were almost the same with each other (Table 4). On the other hand, LEI-9 was 0.9 days longer than LEI-7 (Table 3). These results suggest that the effects of temperature and day length are different on the leaf emergence and the leaf elongation in rice.

In conclusion, we observed whole plant growth using temporally detailed sequential images acquired by HI-RIS Junior. The measurement of each leaf height using these images made it possible to recognize quantitatively the growth process in detail. Regularity of leaf emergence and leaf
elongation are valuable parameters to describe the growth of whole rice plants in tillering stage. Using this method and instrument, the growth process of rice plants could be correlated to specific gene functions in the future. Taken together with mutant study and other molecular biological methods, such systematic and comprehensive phenotypic profiling studies may provide new insights into and advance our understanding of biological procedures in plant growth and development.

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