A Quick Method to Estimate Root Length in Each Diameter Class Using Freeware ImageJ

Ryosuke Tajima\(^1\) and Yoichiro Kato\(^2\)

\(^1\)Field Science Center, Tohoku University, Miyagi 989-6711, Japan;  
\(^2\)Institute for Sustainable Agro-ecosystem Services, the University of Tokyo, Tokyo 188-0002, Japan

Abstract: Digital images for determining root traits have been analyzed using WinRHIZO, commercial software. A freeware ImageJ has been developed for the analysis but whether root lengths assigned to root diameter classes obtained by this method correspond to those estimated by WinRHIZO has not been confirmed. In this study, root lengths estimated using ImageJ were compared with those estimated using WinRHIZO. In ImageJ, the root lengths were estimated using a triangle thresholding algorithm that was valid for total root length in our previous study. For the small-diameter classes (<0.5 mm), the estimates obtained using ImageJ and the conversion factor 2/3, corresponded closely to the estimates obtained using WinRHIZO. For large-diameter classes (>0.5 mm), root lengths estimated using ImageJ were similar to those obtained using WinRHIZO without using the conversion factor. These results were valid for rice roots. In this report, we propose completely automated estimation of root lengths for roots in each diameter class by using ImageJ.

Key words: Image analysis, ImageJ, Root diameter, Root length, WinRHIZO.

Root length and root diameter are important traits for examining nutrient and water uptake from the soil (Lynch, 2007). Recently, estimation of root length and root diameter has been greatly improved by the development of image-processing software and high-resolution image scanners (Bouma et al., 2000; Kaspar and Ewing, 1997; Kimura and Yamazaki, 2001, 2003; Zobel, 2003). The commercial software WinRHIZO (Regent Instruments Inc., Quebec, Canada, 2000) has been the most commonly used program in rice root studies (Kato et al., 2010; Henry et al., 2011). WinRHIZO provides a fairly accurate measurement of root length and root diameter (Bouma et al., 2000), but it is expensive and it takes 5-10 min to analyze one image because special image processing is required even now (i.e., dividing an image into numerous subimages before analysis).

Another accepted protocol for estimating root length and root diameter is to use the new freeware package called ImageJ (formerly NIH Image), developed at the National Institutes of Health (NIH) in the United States (Kimura et al., 1999). In our previous study, root length estimated using ImageJ with the 16 threshold algorithms of image processing for binary images was compared with root length estimated using WinRHIZO (Tajima and Kato, 2011). The study showed that the root length obtained using ImageJ with the triangle algorithm and a conversion factor of 2/3 matched the root length obtained with WinRHIZO. In this study, we used this quick method for root length measurement, developed previously, to determine root length in 6 diameter classes.

Materials and Methods

A field experiment was conducted at the Institute for Sustainable Agro-ecosystem Services (ISAS), University of Tokyo, Tokyo (35°43’ N 139°32’ E) in the summer 2009. Rice (Oryza sativa L.) plants (cv. Takanari) were grown in lowland fields under 3 water management conditions: aerobic, saturated, and flooded. The detailed cultural practices were as described previously (Kato et al., 2010). The samples were washed carefully on a 0.5-mm mesh screen. After removing debris, an 8-bit grayscale image was acquired by digital scanning at a 400-dpi resolution by using an image scanner and a positive film transparency unit (Epson GTX970, Seiko Epson Corp., Nagano, Japan, or Epson Expression 10000XL, Epson America, Inc., San Jose, CA, USA). The root images were saved in TIFF.
format; 42 rice root images were collected for the analyses (file size: about 27 MB/image). The root images were transformed to binary images by using the triangle thresholding algorithm as premeasured using ImageJ (Version 1.42q, NIH, http://rsb.info.nih.gov/ij/index.html), according to our previous study (Tajima and Kato, 2011). The triangle algorithm determines the threshold value by normalizing the height and dynamic range of the pixel intensity histogram of an image (Zack et al., 1977). After obtaining the threshold, we analyzed the binary images by using the line-measured algorithm by Kimura et al. (1999). In the line-measured algorithm, root length is calculated by counting the number of orthogonally and diagonally connected pairs of pixels. Root lengths in each root diameter class were measured as described by Kimura and Yamazaki (2001). Briefly, the peripheral pixels of the binary image were deleted, and root length was estimated. This process was repeated until no pixels remained in the image. Root diameter was estimated using the number of deletions. The same root images were also analyzed using WinRHIZO (Version 2009b, Regent Instruments, Montreal, QC, Canada) in Lagarde’s mode (Bouma et al., 2000). We used linear regression analysis to assess the relationship between ImageJ-estimated and WinRHIZO-estimated root length datasets.

### Results and Discussion

The diameter classifications obtained using ImageJ and WinRHIZO are presented in Table 1. With ImageJ, root diameter estimation was based on the number of deletions (Kimura and Yamazaki, 2001). Therefore, the diameter classification depends on the resolution of the digital

Table 1. Relationship between root diameter classes obtained by ImageJ and WinRHIZO.

<table>
<thead>
<tr>
<th>Root diameter classes (mm)</th>
<th>ImageJ</th>
<th>WinRHIZO</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.07</td>
<td>&lt; 0.1</td>
<td></td>
</tr>
<tr>
<td>0.07 − 0.21</td>
<td>0.1 − 0.2</td>
<td></td>
</tr>
<tr>
<td>0.21 − 0.50</td>
<td>0.2 − 0.5</td>
<td></td>
</tr>
<tr>
<td>0.50 − 0.93</td>
<td>0.5 − 1.0</td>
<td></td>
</tr>
<tr>
<td>&gt; 0.93</td>
<td>&gt; 1.0</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Relationship between the proposed protocol and WinRHIZO-estimated root length in 5 diameter classes. In the proposed protocol, the conversion factor of 2/3 is used for the ImageJ-estimated root lengths in the small-diameter classes (<0.5), and the conversion factor is not used for the root lengths in the large-diameter classes (>0.5). Solid lines are regression lines, dashed lines are 95% confidence intervals, and dotted lines are 95% prediction intervals (n = 42).
image. At a 400-dpi resolution, root diameter class can be estimated at intervals of 0.07 mm. Although the algorithm used for root diameter classification with WinRHIZO was uniform, root length in each diameter class was divided into the following 6 classes: <0.1, 0.1–0.15, 0.15–0.20, 0.20–0.50, 0.50–1.0, and >1.0 mm. In this study, root length in each diameter class was compared between ImageJ and WinRHIZO, as shown in Table 1.

In our previous study (Tajima and Kato, 2011), total root length estimated using ImageJ with the triangle thresholding algorithm showed a good fit with the total root length estimated using WinRHIZO when the conversion factor of 2/3 was used. Root length using the protocol also correlated closely with root length estimated using WinRHIZO when the conversion factor of 2/3 was used. Root length using the protocol also correlated closely with root length estimated using WinRHIZO. For root lengths in the 3 small-diameter classes (<0.1, 0.1–0.2, and 0.2–0.5), the regression slopes were almost identical. However, the root lengths in the 2 large-diameter classes (0.5–1.0 and >1.0) were underestimated, in comparison with those estimated using WinRHIZO. The mean ratio of the ImageJ estimates to the WinRHIZO estimates was 1.50; the ImageJ-estimated root lengths in the large-diameter classes (>0.5) do not require multiplication by the conversion factor (Fig. 1).

Root lengths in the 5 diameter classes obtained using the proposed protocol and total root length as their sum are depicted in Fig. 1. Root lengths estimated by the protocol using ImageJ were correlated with root lengths estimated by WinRHIZO in 5 diameter classes, even though the diameter classifications were different. The regression slopes ranged from 0.84 to 1.16. The difference in the estimates between the 2 methods was ±16%, partly because of the difference in diameter classification between ImageJ and WinRHIZO.

In conclusion, this study showed that the estimation of root length for each diameter class by using the ImageJ triangle thresholding algorithm and the conversion factor of 2/3 for diameters < 0.5 mm and 1.0 for diameters >0.5 mm was an appropriate protocol, as compared with the root lengths estimated using WinRHIZO. The method using ImageJ does not require any manual operation for image processing including the creation of binary images and enables the rapid analysis of rice root lengths (about 50 images hr⁻¹).

Acknowledgements

We thank K. Ichikawa, R. Soga, and K. Yatsuda (ISAS, University of Tokyo) for their technical assistance in conducting these experiments.

References