Easily Accessible Method for Root Length Measurement Using an Image Analysis System

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Received July 29, 1994

Abstract: Convenient methods for root length measurement are highly demanding. In this report we demonstrated an easily accessible method of image analysis for this purpose consisting of a personal computer, a commercially-available image scanner, a public domain image analysis and processing software (NIH Image). Its performance, together with that of the root length scanner, was evaluated by way of a comparison with the directly-measured root length for soybean, maize and rice, all of which differ in root diameter and rooting density of lateral roots. Preliminary trials for the measurement of nylon threads and stainless wires with known length validated the accuracy of this method for the expected range of root diameters. This method measured length precisely for maize and soybean, while the root length scanner read 81% of the actual length for maize and 89% for soybean. For rice, the root system of which consisted very fine roots, the image analysis method gave an underestimation of about 7.5%, probably due to the fact that overlapping and clumping of thin lateral roots was unavoidable during the root sample preparation for image analysis. By contrast, the root length scanner measured only one fourth of the actual length. This method also saved time required for measurement from one-fourth to one-fifth compared with direct measurement.

Key words: Image analysis, Image scanner, NIH Image, Personal computer, Root length, Root length scanner.

Root length is one of the most functionally-significant and thus frequently-measured traits of plant root systems. Since this measurement is quite tedious if done manually, three main convenient methods have been developed so far, and their accuracy and performance have been examined. First, the line-intersect method needs only a few inexpensive instruments, but the accuracy of the data greatly depends on the performance of individuals. Second, the root length scanner is suitable when a large amount of roots need to be measured. But again the data are less reliable and tend to be underestimated, especially when the root system to be measured contains a significant amount of fine roots, specifically those with diameters which are less than 100 \( \mu m \). Third, recently computer-aided image analysis systems have been developed for the measurement, but all of the reported methods require a specifically developed image analysis program and sometimes specifically designed data acquisition equipment. As to the accuracy of the data obtained with those systems, reports have validated the data by comparing it with that obtained with the line-intersect methods and other methods, but not with the actual root length.

In this paper, we showed an easily acce-
sible method for root length measurement by using a personal computer-aided image analysis system where root image data were acquired by a commercially-available image scanner for general purposes and processed with a public domain image analysis program. The performance of this system was evaluated on the roots of three crop species (rice, maize and soybean), which differ in root diameter and lateral root density on main root axis. The accuracy of the data were checked against that obtained by directly measured actual root length and by the root length scanner method.

Materials and Methods

Root sample preparation
Rice (cv. Nipponbare), maize (yellow dent corn, cv. Nagano 1) and soybean (cv. Kingen 1), were planted in root boxes (25 cm × 2 cm × 40 cm) filled with 2.6 kg of loamy sand soil (one plant per box). They were grown in a growth chamber at 30°C with a 16-h photoperiod for 3 weeks. Rice plants were grown under submerged conditions and the others under well-watered conditions.

Root systems were carefully washed out from the root box according to the method proposed by Kono et al.9), which allowed fast root sampling with little damage and loss, and the sampled roots were preserved in FAA (formalin : acetic acid : 70% alcohol (1:1:18). Before measurement, root systems were stained dark blue with 0.25% Coomassie Brilliant Blue R. This procedure is important to get good contrast of root image against background during scanning by the image scanner. Two different small parts in a root system were taken from each plant and three plants were measured for each species. The roots were cut into 2-cm segments and the length was measured with the root length scanner (Commonwealth Aircraft Co. Ltd). Size of each root sample was adjusted to about 5 m in the scanner reading (note that this value substantially differed from the actual length as later described).

Root length measurement with the image analysis system
After measurement, the root samples were all collected and then scattered evenly on nylon mesh floating on water. Special care was taken to avoid overlapping and clumping of roots, especially lateral roots, during this procedure. Nylon mesh was then carefully lifted up from water and turned over on a clear sheet and removed from the clear sheet, leaving only root specimens on the sheet and then excess water was removed from the sheet. The sheet with root segments (10 cm × 15 cm) was then scanned with an image scanner (Epson, GT-6000) at 250 dpi and digitized signals were transmitted to a personal computer (Macintosh IIcX with 20 mega bytes of RAM), where root images (256 gray levels) were stored.

For measurement of root length, NIH Image ver. 1.44 (public domain image processing and analysis program released from the National Institutes of Health, U.S.A.) was used. After enhancing the edges, the gray levels of the root image were adjusted so that all root parts were discriminated from the background with little noise by comparing the image with actual roots, and then the grayscale root image was converted to a binary image. The binary image was skeletonized until the image came to consist of single line of pixels, whose numbers were then counted by the program. Root length was calculated by multiplication of the side length of a pixel and the number of pixels.

Length correction factor
The above calculation gives a precise root length only when the pixels are lined vertically or horizontally. In other cases, for example, when randomly-scattered roots are measured as indicated in Fig. 1, errors are introduced, i.e., the calculated length \(r_c\) is always smaller than the actual length \(r\), and thus correction will be required.

Assuming that the roots, i.e., single pixel lines, are evenly aligned in all directions, increase of \(\theta\) from 0 to \(\pi/4\) corresponds to the increase of \(y\) from 0 to \(r/\sqrt{2}\) where \(\theta\) is an angle between the horizontal line (x axis) and a root, and then the area of the shaded part can be obtained by the following equation:

\[
\int_0^{\pi/4} \sqrt{r^2 - y^2} \, dy
\]

Then the mean of \(r_c\), calculated root length for the ones aligned from 0 to \(\pi/4\) can be obtained by dividing the area by the height which is \(r \sin \pi/4\) as follows:

\[
\frac{1}{r \sin \pi/4} \cdot \int_0^{\pi/4} \sqrt{r^2 - y^2} \, dy = 0.9087r
\]
The reciprocal number of the coefficient of \( r \), 1.10, was used as a correction factor to obtain the corrected root length from the calculated length.

After these procedures, all the root segments on the clear sheet were collected and the length was measured visually by using a ruler (direct measurement). For further validation of this method, length of stainless wire (25 \( \mu m \) in diameter) and nylon thread (500 \( \mu m \) in diameter) with known length were measured by the same manner as root measurement.

**Results and Discussion**

It is evident from Fig. 2 that the actual length of thread and wire could be measured quite precisely by our image analysis method when the above correction factor is considered. And this held regardless of the diameter of objects. In general, the difficulties involved in root length measurement are caused mainly by the great number and small diameter of lateral roots that account for a major part of the total root length. Lateral root diameters of the three species examined in this study mostly fall in this range (25 to 500 \( \mu m \)). Therefore, if special attention is paid during root sample preparation so that numerous lateral roots can be separately recognized as digitized images, this method should give precise root length values.

Table 1 compares the performance of the image analysis method with the root length scanner method. The values of each method are expressed as a percentage to the actual length measured by the direct method.

<table>
<thead>
<tr>
<th>Species</th>
<th>Root length scanner</th>
<th>Image analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean</td>
<td>89.1±9.5</td>
<td>99.9±3.5</td>
</tr>
<tr>
<td>Maize</td>
<td>81.3±7.5</td>
<td>99.0±3.1</td>
</tr>
<tr>
<td>Rice</td>
<td>24.3±2.2</td>
<td>92.5±3.9</td>
</tr>
</tbody>
</table>

Data are the mean of six measurements and S.D.
of the actual length. This was caused by the fact that a rice root system mainly consisted of fine lateral roots. For example, a one-month-old rice plant formed a root system of about 178 m in length, and 75% of the length was accounted for by the non-branching 1st order lateral roots and 2nd and 3rd order lateral roots, most of which were less than 100 μm in diameter.

In contrast, the image analysis method showed high accuracy also for rice roots, but it still slightly underestimated. This underestimation could most probably be attributed to the difficulty in avoiding the overlapping and clumping of lateral roots due to their high density during sample preparation for image analysis. Average lateral root density (no./cm main roots axis) was 4.6 for soybean, 6.8 for maize and 14.6 for rice. Because of this high density of lateral roots in rice, some of them could possibly have overlapped and attached to one another.

In conclusion, we could obtain root length, which is practically precise enough with our image analysis method, for crop species with roots of different diameters. This method saved measurement time, reducing it to one-fourth to one-fifth compared with a direct measurement. And about 90% of the time had to be used for root sample preparation, which was critical for precise measurement, especially for rice. Therefore, this method is quite useful, especially when length of roots with small diameter needs to be measured precisely. Combination of this method and the root length scanner, which is suitable for the measurement of large amount of roots with a relatively large diameter, depending on the research objectives, will provide a strong tool for effective root length measurement.

Acknowledgment

We are grateful to the Japanese Ministry of Education, Science and Culture for its support in carrying out this investigation under Grant No. 05304012.

References