A Nonparametric Test on Differences in Growth Direction of Rice Primary Roots*

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Abstract: Sometimes there is a need to examine the spatial distribution of roots in studies of rice plants. For analysis of growth direction of primary roots (GDR), a statistical method requiring an assumption of a given distribution is not available, because the distribution pattern of GDR is not always known. A nonparametric method will be useful for analysis of such an unknown distribution. We tested the difference in distribution of GDR with Kolmogorov-Smirnov two sample test method among three samples of rice cultivars, Nankin 11 (A), Dobashi 1 (B) and Mubo-aioku (C). The relative cumulative frequency distribution of GDR (Sn(x)) was calculated for each sample. When the maximum absolute difference of Sn(x) was larger than the critical value (Dα) between two samples, we concluded that the two samples have significantly different distributions of GDR at the level of α. The result of the test indicated a difference in distribution of only A from that of B or C, though it seemed in histogram that B had a distribution pattern of GDR intermediate between those of A and C. That suggests the effectiveness of nonparametric methods for precise analysis of GDR.

Key words: Kolmogorov-Smirnov two sample test, Nonparametric method, Oryza sativa L., Rice plant, Root distribution.

In studies of root development, morphological characters of primary roots are examined. Sometimes there is a need to compare those characters between individuals. However, the distribution** of root characters cannot always be described by any given distribution pattern (e.g. the normal in ANOVA), because primary roots of one plant interact one another in an unknown way. In such a case, some distribution-free method are required for statistical analysis.

In this paper, we present a use of nonparametric method to test differences in growth direction of primary roots between rice plants.

Materials and Methods

Cultivation of rice plants was carried out in an experimental field of the University of Tokyo (Bunkyo-ku, Tokyo) in 1985. Three rice cultivars, Nankin 11 (A), Dobashi 1 (B), and Mubo-aioku (C), were grown in flooded 1/2000a Wagner Pots. Plants were taken at heading stage. The growth direction of their
primary roots (GDPR), which means angles of primary roots to the horizon, were estimated by the method of Morita et al\textsuperscript{21}.

The differences between any two samples were tested with Kolmogorov–Smirnov two sample test\textsuperscript{13,4}. The test is based on differences between the relative cumulative frequency distributions of the sample \((S_n(x))\). Initially differences between A and B were tested as follows (Fig. 1):

The null hypothesis is that the two samples were taken from populations with the same distribution, namely, the distribution of GDPR in A and B are not different from each other. If the maximum absolute difference between \(S_{\lambda}(x)\) and \(S_{\mu}(x)\) \((D_{\lambda\mu})\) is larger than the critical value \(D_{a}\), the null hypothesis will be rejected at the level of \(\alpha\). When sample sizes are large enough, we can determine \(D_{a}\) as:

\[
D_{a} = K_{a} \sqrt{\frac{n_{\lambda} + n_{\mu}}{n_{\lambda} n_{\mu}}} \tag{1}
\]

where \(K_{a} = \sqrt{\frac{1}{2} \left(-\ln \frac{\alpha}{2}\right)} \tag{2}\)

The difference between other couples among the samples (i.e. A–C and B–C) were tested in the same way.

**Results**

The numbers of primary roots of A \((n_{\lambda})\), B \((n_{\mu})\) and C \((n_{\nu})\) were 685, 922 and 1025 respectively. The \(S_{n}(x)\)'s are shown in Table 1. The maximum differences of \(S_{n}(x)\) among the samples were compared with \(D_{a}\) calculated on the basis of formula 1 and 2 (Table 2).

Since \(D_{AB} = 0.103\) was larger than \(D_{0.05} = 0.082\) (Table 2), we rejected the null hypothesis and concluded that the distribution of GDPR of A and B were different from each other. A similar conclusion was made for the difference between A and C. On the other hand, we could not reject the identity of the distributions between B and C, because \(D_{BC}\)

![Table 1. The relative cumulative frequency distributions of the growth direction of primary roots (GDPR).](image)

<table>
<thead>
<tr>
<th>GDPR (degree)</th>
<th>Relative cumulative frequency distribution (S_{n}(x))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(S_{n}(x))</td>
</tr>
<tr>
<td>10</td>
<td>0.015</td>
</tr>
<tr>
<td>20</td>
<td>0.041</td>
</tr>
<tr>
<td>30</td>
<td>0.147</td>
</tr>
<tr>
<td>40</td>
<td>0.339</td>
</tr>
<tr>
<td>50</td>
<td>0.620</td>
</tr>
<tr>
<td>60</td>
<td>0.836</td>
</tr>
<tr>
<td>70</td>
<td>0.931</td>
</tr>
<tr>
<td>80</td>
<td>1.000</td>
</tr>
<tr>
<td>90</td>
<td>1.000</td>
</tr>
</tbody>
</table>

![Fig. 1. A flow chart showing the procedure of the test.](image)
Table 2. The absolute difference of relative cumulative frequency distributions of the growth direction (S(x)).

<table>
<thead>
<tr>
<th>GDPR (degree)</th>
<th>Difference of S(x)</th>
<th>A-B</th>
<th>A-C</th>
<th>B-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.000</td>
<td>0.004</td>
<td>0.004</td>
<td>0.004</td>
</tr>
<tr>
<td>20</td>
<td>0.008</td>
<td>0.004</td>
<td>0.004</td>
<td>0.004</td>
</tr>
<tr>
<td>30</td>
<td>0.005</td>
<td>0.011</td>
<td>0.016</td>
<td>0.016</td>
</tr>
<tr>
<td>40</td>
<td>0.054</td>
<td>0.030</td>
<td>0.024</td>
<td>0.024</td>
</tr>
<tr>
<td>50</td>
<td><strong>0.103</strong></td>
<td>0.098</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td>60</td>
<td>0.081</td>
<td><strong>0.127</strong></td>
<td><strong>0.046</strong></td>
<td>NS</td>
</tr>
<tr>
<td>70</td>
<td>0.049</td>
<td>0.087</td>
<td>0.038</td>
<td>0.038</td>
</tr>
<tr>
<td>80</td>
<td>0.001</td>
<td>0.000</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>90</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Critical value

D_{05} = 0.069, 0.067, 0.062
D_{01} = 0.082, 0.080, 0.074

The minimum differences (D) are underlined. **Significant difference at 1% level.
NS No significant difference at 5% level.

was smaller than D_{05}, indicating no significant difference at 5% level (Table 2).

Discussion

Instead of parametric methods like ANOVA, histograms are often compared between samples in studies of root development. In this work, the distribution pattern of B seemed to be intermediated between those of A and C in the histogram of GDPR (Fig. 2). However, the result with Kolmogorov–Smirnov two-sample test showed that only GDPR distribution of A is significantly different from the others. That demonstrates the effectiveness of this nonparametric method to accurately determine differences in GDPR distribution.

The Kolmogorov–Smirnov two-sample test only shows if there is a difference between two samples and gives no information as to ‘how’ those two distributions differ from each other. Meanings of the difference can be supposed by comparison of averages, medians, or some other statistical values of the data. We recommend to use a combination of this method and some basic statistical method for analysis of GDPR.

In the present study, we tested the difference on the basis of relative cumulative frequency distributions (S(x)) by 10 degree-increments. Practically, however, some larger and/or irregular intervals are acceptable, unless the apparent value of D is much smaller than the real value of D. For example, the relative cumulative frequency distributions by 20 degree-increments gave similar results in this study.

This method will also be useful to test the fit of a simulated distribution of GDPR or other characters of rice roots.

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* In Japanese with English summary.