Variability of Root Anatomical Traits in Rice Germplasm

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ABSTRACT

Root traits influence the amount of water and nutrient absorption, and are important for maintaining crop yield under drought conditions. The objectives of this research were to characterize variability of root traits among rice genotypes. Plants were grown in 150 cm columns for 45 days in a greenhouse under optimal growth conditions. Root diameter, Cortex diameter, Stele diameter, late meta-xylem diameter and number of xylem were determined for 268 genotypes of the rice germplasm. Significant genetic variability was observed for root anatomical traits among rice genotypes in Indica panel. Five quantitative characters i.e. root cross section diameter, root cortex diameter, stele diameter, late meta-xylem diameter and late meta xylem number were measured. The largest variation was observed for late meta-xylem number with Coefficient of Variation (CV) of 11.56% followed by stele diameter, late meta-xylem diameter, root cortex diameter and root cross section diameter. Ratio of stele diameter to root diameter has shown the least variation with the CV of 6.51%. The trait such as stele diameter, late meta-xylem diameter, late meta-xylem number and ratio of stele diameter to root diameter has recorded as leptokurtic. All the traits registered as positively skewed. The genetic variability identified in this research for root traits can be exploited to improve drought tolerance and/or resource capture in rice.

Key words Root anatomy, coefficient of variation, skewness, kurtosis and rice

Among those stresses, drought is the most widespread limitation to rice productivity under dry-land conditions. Consequently, developing drought-tolerant rice genotypes has been the focus of many rice improvement programs. Root traits are critical for soil exploration and water and nutrient uptake, and are important for crop improvement under drought conditions (Manschadi, et al., 2008). Increased root diameter was associated with drought tolerance in rice (Oryza sativa L.) because thicker roots have large xylem vessels with increased axial conductance and are more efficient in penetrating deep soil layers to extract water (Fukai and Cooper, 1995), (Clark et al., 2008).

Despite the importance of root traits in drought tolerance, little work has been done to include drought-adaptive root traits in breeding for drought-tolerant rice varieties. Most rice improvement programs have concentrated on above-ground components, particularly for decreasing plant height and increasing harvest index. Crop breeding programs have largely ignored root traits, mainly because of the difficulties associated with root recovery and evaluating root traits in situ. In addition, large phenotypic plasticity of root traits in response to changes in soil conditions, and lack of high-throughput and cost-effective screening techniques make root studies highly challenging (Manschadi, et al., 2008), (Poorter and Nagel, 2000), (Fitter, 2002). As a result, limited information is available on genetic variability of root traits in rice. Exploring genetic variability of root traits could assist rice improvement programs in developing varieties with desired root traits for drought tolerance or target environments. An understanding of the relationship of root traits to the shoot traits that contribute to grain yield is also essential to achieve improvements in productivity.

Rice (Oryza sativa L.) is one of the most important food crops in the world in terms of the area harvested, production, and productivity (FAO, 2011). Wheat is grown in a wide variety of environments from tropical to temperate. Although rice has a wide range of climatic adaptability, its productivity is limited by several abiotic stresses.
The study aimed to determine level of germplasm variation to identify and classify variation for grouping the accessions by taking into account several characteristics and relationship between them.

**MATERIALS AND METHODS**

The germplasm collection consisting of 268 rice accessions was used in this study, which consist of land races and varieties collected from gene bank, International Rice Research Institute, Manila, Philippines. All accessions were grown in Greenhouse with controlled environment. Dormancy of rice seeds was broken after exposure to 50°C for 3 days and pre-germinated seeds were sown in white-colored painted pots (55 cm long and 15 cm diameter) as recommended in (Poorter et al., 2012) to minimize the confounding effects of increasing temperature of pot surface and soil. The pots were filled with 11 kg of clay loam soil and maintained under natural greenhouse conditions at IRRI during the 2012 wet season i.e., during the season when temperature in the greenhouse and pot can be controlled best. Each pot was drilled with holes on either side at the bottom for imposing controlled water-deficit stress and lined with polythene covers to facilitate easier separation of roots from soil at the end of the treatment.

**Water-deficit stress imposition**

Rice plants were maintained at two moisture regimes: control at 100% field capacity (FC) that is the maximum soil moisture content after drainage of excess water resembling an aerobic condition and water-deficit stress at 55-60% FC. Water deficit stress was imposed after seedling establishment, that is, 15 days after seedling emergence, before which all the pots were maintained uniformly at 100% FC. Pots with the control treatment were maintained at 100% FC throughout the experiment while water-deficit stress was imposed by unplugging the stoppers at the bottom of the pots. A standardized gravimetric approach of daily pot weighing (Raju, et al., 2014) was followed for 30 days to gradually attain 55-60% FC and thereafter maintained at the same level until the end of the experiment Once the target stress level was reached, daily consumed water due to transpiration was replenished by adding an exact amount of water to bring back the moisture content to the desired target in each pot. The soil surface was covered with a circular polythene sheet to protect from direct evaporative loss of water and a slit across the radius of the polythene sheet prevented heat buildup on the soil surface. Daily pot weights recorded for 30 consecutive days of stress period.

**Root sample processing**

The entire column of soil along with the roots was placed on a 1 mm sieve and meticulously washed using a gentle stream of water to minimize the loss of small roots and root hairs. Rice root system is mainly composed of nodal roots and only one radicle or seminal root (primary root), with the latter growing to a maximum length of 15 cm and being viable until 7 leaf stage. To make meaningful comparison nodal root was investigated in our study. Across 268 rice accession, roots were collected from near the root shoot junction (RSJ) (Henry et al., 2012). Collected samples were stored in 40% alcohol to study root anatomy.

**Table 1. Characteristic means and variations of 268 accessions**

<table>
<thead>
<tr>
<th>Variability index</th>
<th>Root Dia. (µm)</th>
<th>Cortex dia. (µm)</th>
<th>Stele dia. (µm)</th>
<th>Late meta xylem dia. (µm)</th>
<th>Number of late metaxytem</th>
<th>SD:RD ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum</td>
<td>240587.32</td>
<td>74733.14</td>
<td>61149.05</td>
<td>11123.16</td>
<td>1261.00</td>
<td>6842.57</td>
</tr>
<tr>
<td>Mean</td>
<td>897.71</td>
<td>278.85</td>
<td>228.17</td>
<td>41.50</td>
<td>4.71</td>
<td>25.53</td>
</tr>
<tr>
<td>SD</td>
<td>79.73</td>
<td>26.85</td>
<td>24.31</td>
<td>4.23</td>
<td>0.54</td>
<td>1.66</td>
</tr>
<tr>
<td>CV</td>
<td>8.88</td>
<td>9.63</td>
<td>10.65</td>
<td>10.18</td>
<td>11.56</td>
<td>6.51</td>
</tr>
<tr>
<td>Skewness</td>
<td>0.13</td>
<td>0.02</td>
<td>1.62</td>
<td>1.18</td>
<td>1.14</td>
<td>1.22</td>
</tr>
<tr>
<td>Kurtosis</td>
<td>0.20</td>
<td>-0.22</td>
<td>5.57</td>
<td>3.95</td>
<td>3.57</td>
<td>3.41</td>
</tr>
<tr>
<td>Min.</td>
<td>673.00</td>
<td>202.56</td>
<td>181.12</td>
<td>31.42</td>
<td>3.50</td>
<td>21.64</td>
</tr>
<tr>
<td>Max.</td>
<td>1167.62</td>
<td>345.41</td>
<td>344.10</td>
<td>65.31</td>
<td>7.50</td>
<td>33.46</td>
</tr>
</tbody>
</table>
Fig. 1. Frequency distribution of different root anatomical traits in rice germplasm.
Root anatomy

To investigate root anatomical features, samples stored in 40% ethanol obtained from the root shoot junction were hand-sectioned with a razor blade under a dissecting microscope. Root sections were stained with 0.5% w/w phloroglucinol in water followed by 20% (V/V) HCL (Jensen, 1962) for lignin staining. Images of the root sections were acquired with a Zeiss Axioplan 2 compound microscope (Zeiss, Germany) with 50× and 100× magnification. At least 3-5 root images were considered for measuring anatomical traits such as root cross-section diameter, cortex diameter, stele diameter and late metaxylem diameter, with image J software (Abramoff et al., 2004).

RESULTS AND DISCUSSION

The First order Statistical measures i.e. maximum, minimum, sum, mean, Standard Deviation (SD) and Coefficient of Variation (CV) for the measured traits are presented in Table 1. The largest variation was observed for number of late meta-xylem (LMXN) with CV of 11.56% followed by stele diameter (SD) with CV of 10.65%, Late meta-xylem diameter (10.18%), root cortex diameter (9.63%) and root cross section diameter (8.8%) Stele diameter (SD): Root diameter (RD) ratio has shown the least variation with the CV of 6.51%.

The overall mean of root cross section diameter was 897.71µm. The skewness and kurtosis coefficient were 0.13 and 0.20 respectively. The minimum and maximum value observed for root cross section diameter was 673 µm and 1167.62 µm respectively. The root cross section has shown the coefficient of variation with the value of 8.88% and standard deviation 79.73. The root cortex diameter has shown the mean value of 278.85 µm. This trait exhibited negative kurtosis coefficient with the value of -0.22. The coefficient of variation recorded for root cortex diameter was 9.63%. The skewness coefficient of root cortex diameter recorded least value with 0.02. The minimum and maximum value recorded for this trait was 202.56 µm and 345.41 µm respectively.

The wide range of variation was observed in stele diameter with the mean of 228.17 µm. It also showed coefficient of variation was 10.65%. The highest value of skewness and kurtosis coefficient was recorded as 1.62 and 5.57 respectively. The maximum and minimum value observed for stele diameter was 344.10µm and 181.12µm respectively.

The maximum value of late meta-xylem diameter was recorded as 65.31µm. The mean of late meta-xylem diameter for 268 germplasm lines were 41.50 µm with the skewness and kurtosis coefficients of 1.18 and 3.95. The late meta-xylem diameter recorded the coefficient of variation was 10.18%. This trait has shown the minimum value of 31.42µm. The mean of late meta-xylem number was recorded as 4.71 µm and this trait exhibited highest coefficient of variation with the value of 11.56%. The skewness and kurtosis coefficient was observed with the value of 1.14 and 3.57 respectively. The maximum and minimum value of late meta-xylem number was recorded as 7.5 and 3.5 respectively.

The least variation observed for ratio of stele diameter to root diameter with coefficient of variation was 6.51%. It was registered with the mean of 1.66. The coefficient of skewness and kurtosis was 1.22 and 3.41 respectively. It had a maximum value of 33.46 and minimum value of 21.64. The accessions possessing extreme phenotype can be utilized in mapping population development for Quantitative trait loci identification. Coefficient of variation was high for all the traits measured. The study of distribution of quantitative traits using skewness and kurtosis provides information about nature of gene action (Fisher et al., 1932) and number of genes controlling the traits (Robson, 1956) respectively.

The skewed distribution of a trait in general suggests that the trait is under the control of non-additive gene action and is influenced by environmental variables. Positive skewness is associated with complementary gene interactions while negative skewness is associated with duplicate (additive x additive) gene interactions. The genes controlling the trait with skewed distribution tend to be predominantly dominant irrespective of whether they have increasing or decreasing effect on the trait (Pooni, et al., 1977). Frequency distribution for different traits on 268 germplasm accessions revealed different patterns of distribution as shown on Figure 1. None of the traits had shown the normal distribution. All traits were showing positive skewness. If skewness is positive, the data are positively skewed or skewed right, meaning that the right tail of the distribution is longer than the left. If skewness is negative, the
data are negatively skewed or skewed left, meaning that the left tail is longer (Joanes and Gill).

Kurtosis is negative or close to zero in the absence of gene interaction and is positive in the presence of gene interactions (Kotch et al., 1992). The traits with leptokurtic and platykurtic distribution are controlled by fewer and large number of genes respectively. As none of the traits had kurtosis coefficient of 3 or mesokurtic, they were not in normal distribution. The traits stele diameter, late meta-xylem diameter, late meta-xylem number and ratio of stele diameter to root diameter were leptokurtic. The traits root cross section diameter and root cortex diameter were platykurtic.

The genetic variability identified in this research for root anatomical traits can be exploited to improve drought tolerance and/or resource capture in rice. Our future research will evaluate drought tolerance of the contrasting genotypes identified in this study for root traits under controlled environment and field conditions.

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LITERATURE CITED


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