Effects of Aeration on Root Physiology and Nitrogen Metabolism in Rice

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Abstract: In order to clarify the effects of aeration on root nitrogen metabolism in rice seedlings, rice cultivars Guodao 6 (indica) and Xiushui 09 (japonica) were investigated for root growth, the activities of glutamine synthetase (GS), glutamic acid-pyruvic acid transaminase (GPT) and glutamic acid oxaloacetate transaminase (GOT), the nitrate (NO₃-N) concentration, the contents of free amino acids and soluble sugar in root under hydroponics with continuous aeration treatment. The results showed that rice seedlings grown in oxygenation solutions had higher root dry matter, longer root length, stronger root activity and larger root absorption area compared with the control. In addition, the contents of soluble sugar, root vigor and the activities of GS, GOT and GPT in the aeration solutions were higher than those in the control. The results also indicated that the activities of enzymes involved in root nitrogen metabolism of Xiushui 09 were enhanced by aeration, however, there was no significant influence on root nitrogen metabolism of Guodao 6, which suggested that effect of oxygenation on rice root nitrogen metabolism might be genotype-specific.

Key words: rice; aeration; root physiology; nitrogen metabolism

Root is an important organ of a plant which obtains water, nutrients and oxygen from the soil (Sun et al., 2002). As a result, research on the morphology and physiology of root, including the development, response to environmental variables and so on, are of great significance. Relevant studies involve rice root development, morphology, physiology, ecology, genetics and other fields, the relationships between growth, distribution, physiological activity and production, response to environmental factors and other fields have also made great progress (Ling and Lu, 1984; Schiefelbein et al., 1991; Karroy, 1994; Shi et al., 1997; Thaler and Pages, 1998; Li et al., 2001; Williamson et al., 2001; Mian et al., 2003; Ye et al., 2005). Although rice is adapt to grow in flooded conditions, the oxygen concentration in the mud plays a crucial role in the morphogenesis and nutrient absorption of rice root (Gibbs and Greenway, 2003; Morard et al., 2004). Additionally, nitrogen metabolism is an important physiological process for plants (Gang et al., 2010), which affects plant metabolism and development (Li et al., 1993; Scheibl et al., 2004). Due to the direct relationship between crop shoot biomass and economic yield, previous studies have mainly focused on shoot nitrogen metabolism, while few on root nitrogen metabolism. In addition, research on root nitrogen metabolism in rice is mainly focused on the amount of nitrogen fertilizer, applying time and the later growth stage (Ning et al., 2009). Our previous studies showed that oxygen has a unique role in the processes of rice growth and nitrogen utilization, which is conducive to nitrogen absorption and utilization in rice shoot (Zhao et al., 2011). However, the effect of oxygenation on nitrogen metabolism in rice root is still unclear. To illustrate the effect of oxygenation on the uptake of nutrient elements by root and possible mechanisms for high yielding, we investigated root characteristics and key enzyme activities of rice in the process of nitrogen metabolism under conventional and high-oxygen solutions.

MATERIALS AND METHODS

Rice materials and growth conditions

The experiments were carried out from April to May, 2011 in a greenhouse at the China National Rice Research Institute, Hangzhou, China. Seeds of indica
hybrid rice Guodao 6 and conventional japonica rice Xiushui 09 were used.

All the seeds were sterilized with 75% ethanol solution and soaked for 2 d. Seeds were germinated at 30 °C and then transferred to sand beds. Subsequently, the seedlings were watered by nutrient solution without nitrogen. The experiment was conducted at 25 °C day/15 °C night temperatures and 70% relative humidity. Supplementary artificial light was provided with incandescent lamps with light intensity of 100 μmol/(m²·s) during overcast days. Twenty-day-old seedlings were transplanted into plastic bucket (30 cm × 20 cm × 30 cm) covered by a PVC plastic board with drilled holes, into which the seedlings were secured with sponge, and the interval between neighboring hole was 20 cm. When new roots appeared (after about 2–3 d in tap water), the seedlings were transferred to nutrient solutions prepared according to the formula of the International Rice Research Institute (IRRI), and the nutrient solutions were refreshed every 5 d. The pH value of the nutrient solution was adjusted with 1 mol/L HCl or NaOH everyday and maintained between 5.5 and 6.0.

There were two treatments: aeration and control. Aeration: Aerator pump (LP-60, Day & Johnson, Shenzhen, China) continued to fill the root zone with air (oxygen inflatable air velocity was 5 L/min), and dissolved oxygen was saturated to simulate the aerobic treatment of the root zone; Control: conventional hydroponics, dissolved oxygen content maintained at 1.0–5.0 mg/L. Each experimental treatment consists of ten pots and three replicates.

After three weeks, the rice seedlings were harvested to determine the root growth, root activity, the activities of glutamine synthetase (GS), glutamic acid-oxaloacetate transaminase (GPT) and glutamic acid-pyruvic acid transaminase (GOT), free amino acid content, soluble sugar content and nitrate concentration.

Dissolved oxygen and pH

Dissolved oxygen in the nutrient solution was determined with a portable dissolution oxygen meter (HI9143, HANNA Instrument, Italy). The pH was measured with a portable pH meter (HI8424, HANNA Instrument, Italy).

Biomass and soluble sugar content

The seedlings were sampled at three weeks after treatment, and separated into shoots and roots. The shoot and root dry weights were determined after drying the samples at 105 °C for 2 h, and at 80 °C to constant weights in an oven. Dry plant samples were ground into powder and sieved through a 2.36-mm nylon sieve. Anthrone method was used to determine soluble sugar content (Qiao, 2002).

Root characteristics and root activity

Roots of Guodao 6 and Xiushui 09 were collected at three weeks after treatment and washed with tap water. The root number and the maximum root length were measured by manual, while root volume and root activity were determined with the $\alpha$-naphthylamine oxidation method as described by Qiao (2002). Root absorption area was determined by the methylene blue staining method (Qiao, 2002).

Root glutamine synthetase (GS), glutamic acid oxaloacetate transaminase (GOT) and glutamic acid-pyruvic acid transaminase (GPT) activities, free amino acid content and nitrate concentration

The GS activity and free amino acid content were determined as described by Zou (2000). The activity of GS was measured in vitro as a synthetase reaction by the formation of $\gamma$-glutamyl hydroxamate. One unit of enzyme is defined as the amount of enzyme that catalyzed the synthesis of 1 μmol of $\gamma$-glutamyl hydroxamate per hour at 37 °C (Zou, 2000).

The GPT and GOT activities were determined as described by Wu et al (1998). The enzyme activities were expressed by the amount of pyruvic acid generated from 1 g sample in 30 min.

Nitrate concentration was determined as described by Tachibana and Konishi (1991). A certain quantity of fresh sample were weighed and put into a test tube after the roots were shredded, cooked for 30 min in a constant temperature water bath and cooled down immediately with ice water. The extract was washed and filtered repeatedly, then 0.1 mL of filtrate and 0.4 mL of 5% salicylic acid-sulfuric acid solution were mixed, standing for 20–30 min (color), then 9.5 mL of 2 mol/L NaOH solution were added and mixed to measure absorbance at 410 nm by a spectrophotometer (752, Shanghai Spectrum Instrument Co. Ltd, China) after cooling.

Statistical analysis

Data from the experiment were analyzed with the Student’s $t$-test ($P = 0.05$) using SPSS 11.5 (SPSS, Chicago, IL, USA).
Table 1. Effect of oxygenation.

<table>
<thead>
<tr>
<th>Item</th>
<th>Guodao 6</th>
<th></th>
<th>Xiushui 09</th>
<th>Oxygenation</th>
<th>Control</th>
<th>Difference</th>
<th>Oxygenation</th>
<th>Control</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root dry weight (g/pot)</td>
<td>4.18</td>
<td>3.53</td>
<td>0.65 a</td>
<td>1.62</td>
<td>1.12</td>
<td>0.50 a</td>
<td>1.14</td>
<td>0.97</td>
<td>0.17 b</td>
</tr>
<tr>
<td>Radicular absorption region (m²/g)</td>
<td>25.53*</td>
<td>12.80</td>
<td>12.73 a</td>
<td>11.38</td>
<td>9.97</td>
<td>1.40 b</td>
<td>12.34</td>
<td>14.80</td>
<td>2.46 b</td>
</tr>
<tr>
<td>Root length (cm)</td>
<td>25.96*</td>
<td>14.29</td>
<td>11.67 a</td>
<td>27.14*</td>
<td>12.34</td>
<td>14.80 b</td>
<td>138.22*</td>
<td>164.89</td>
<td>26.67 b</td>
</tr>
<tr>
<td>Root number (No./pot)</td>
<td>208.44*</td>
<td>254.44</td>
<td>46.00 a</td>
<td>138.22*</td>
<td>164.89</td>
<td>26.67 b</td>
<td>114.79</td>
<td>110.22</td>
<td>4.56 b</td>
</tr>
<tr>
<td>Root volume (mL)</td>
<td>23.50*</td>
<td>16.44</td>
<td>7.06 a</td>
<td>8.00*</td>
<td>5.56</td>
<td>2.44 b</td>
<td>114.79</td>
<td>110.22</td>
<td>4.56 b</td>
</tr>
<tr>
<td>Intensity of root α-naphthylamine oxidation [µg/(g·h)]</td>
<td>130.02*</td>
<td>101.05</td>
<td>28.97 a</td>
<td>114.79</td>
<td>110.22</td>
<td>4.56 b</td>
<td>114.79</td>
<td>110.22</td>
<td>4.56 b</td>
</tr>
</tbody>
</table>

*, Significant difference between the two treatments by t-test at 5% level. Data followed by different lowercase letters present significant difference between two varieties by t-test at 5% level.

RESULTS

Impact of oxygenation on root growth in rice

The effect of root aeration treatment on root growth of rice seedlings was shown in Table 1. Compared to the control, the rice root dry matter accumulation of Xiushui 09 under oxygenation was significantly increased by 44.64%, whereas that of Guodao 6 increased only by 18.40%. There were no significant differences between the two varieties. Rice radicular absorption region is related to root oxygen content. After aeration, the root radicular absorption area of Guodao 6 was 1.99-fold higher than that of the control, while only 1.13-fold for Xiushui 09. Aeration increased rice root length and root volume, but reduced root number significantly. There were significant differences in the root activity of Guodao 6 but not in that of Xiushui 09. In comparison with the control, the root oxidation intensity of Guodao 6 increased by 28.67% under aeration treatment, while that of Xiushui 09 increased only by 0.41%. There were significant differences in the effects of oxygenation on radicular absorption region, root length, root number and root volume between the two varieties.

Effects on glutamine synthetase (GS) activity in rice roots

The effects of oxygenation on rice root GS activity in different genotypes are shown in Fig. 1. Aeration could increase the root GS activity of Xiushui 09 by 48.74% compared with the control, with significant difference; whereas there were no significant differences in Guodao 6 between different treatments.

Effects on glutamic acid oxaloacetate transaminase (GOT) and glutamic acid-pyruvic acid transaminase (GPT) activities

As shown in Fig. 2, there were no significant differences in GOT activity of Guodao 6 under different treatments. However, root GOT activity of Xiushui 09 under aeration treatment was increased significantly. The effect of oxygenation on rice GPT activity was related to rice genotypes, root GPT activity of Guodao 6 under aeriation was increased by 12.26%, while that of Xiushui 09 was reduced by...
20.16% compared with the control (Fig. 3). However, there were no significant differences in the effect of oxygenation on GPT activity for both the two varieties.

**Effects on nitrate concentration**

Aeration had different effects on rice root nitrate concentration in different genotypes (Fig. 4). Root nitrate concentration of Guodao 6 after aeration was 2.58-folds higher than that in the control, while the root nitrate concentration of Xiushui 09 decreased to 83.95% after aeration treatment. There were significant differences between different treatments in both the two varieties.

**Effects on free amino acids in rice roots**

Amino acids are both substrate and important product of nitrogen metabolism, and the changes of free amino acids reflect the intensity of nitrogen metabolism. Free amino acid content of Guodao 6 increased slightly after aeration, and there were no significant differences between treatments (Fig. 5). Compared with the control, root free amino acid content in Xiushui 09 increased by 55.32% in oxygenation treatment, and there were significant differences between treatments.

**Effects on soluble sugar content in rice roots**

As shown in Fig. 6, under the aeration treatment, root soluble sugar content of indica hybrid rice Guodao 6 was increased by 9.43%, while that of Xiushui 09 under aeration was decreased by 1.95%, slightly lower than the control. There were no significant differences between the treatments in both the two varieties.
DISCUSSION

Rice root is a vital organ which absorbs nutrients and water, and is the space of matter assimilation and synthesis (Akira, 2001). The growth of rice needs oxygen. Pezeshki (1996) detected soil oxygen concentration in fields and the results showed that: if soil oxygen concentration in the paddy soil is maintained at least 3%-5% of the oxygen in the air, it can ensure the normal absorption of oxygen in rice roots. Under adverse environmental conditions, such as surface flooding or ice, rice roots are in relatively low oxygen or anaerobic state, so that their morphology and function are greatly affected (Mcdonald et al, 2001; Malik et al, 2003; Armstrong et al, 2005). Deng et al (2009) have found that if oxygen in rhizosphere is rich, the number of rice seedling roots would reduce, and the root absorption area would increase. Xu et al (2007) and other studies have shown that root biomass below 10 cm under intermittent irrigation (aerobic) after heading was higher than that under flooding irrigation treatment (hypoxia). Rice root reached 55–60 cm depth below the soil surface under intermittent irrigation treatment, and 50–55 cm under flooding irrigation, indicating that aerobic cultivation promoted longitudinal growth of rice root, which is consistent with the results of this study. In the present study, aeration significantly increased root length and the root volume but decreased the root number compared with the control, but the increased significantly, which might due to greater increase of root dry matter accumulation under aerobic treatment than the control.

Liesack et al (2000) have found that rice can secrete a few strong oxidizing oxygen (the main form of H$_2$O$_2$) into the surrounding environment in condition of enough oxygen content, which can oxidize reducing state substances around the rhizosphere to oxidation ones; in contrast, in the anaerobic environment, oxygen secreted by root decreases, and arises denitrification and iron-sulfur reduction phenomenon, resulting in poor root development or even rot, seriously affecting root absorption function. The result of this study that root absorption area and oxidation intensity of rice root under aeration increased compared with the control can fully illustrate this point, but the increase extent is related to rice genotype. Under this experimental condition, the increase extent of indica rice Guodao 6 is larger than that of japonica rice Xiushui 09, which may be associated with more root number and larger volume in indica rice Guodao 6.

In the study, dissolved oxygen in the solution was saturated after aeration, and ammonium nitrogen in the solution was soon converted to nitrate nitrogen without the nitrification inhibitor, which leads to the obvious increase of nitrate concentration of rice root under aeration, and the root nitrate concentration of japonica rice was higher than that of indica rice. Duan et al (2004) considered that the presence of NO$_3^-$ could improve the activities of nitrogen assimilation enzymes, mainly glutamine synthetase. Therefore, rice root GS activity after aeration was higher than the control, and the root GS activity of Xiushui 09 increased more significantly than that of Guodao 6. Sugar involves in carbon metabolism, and higher sugar content will be beneficial to root respiration metabolism, providing energy for the physiological activities of roots, and consequently promotes root development. Root soluble sugar content was significantly increased after aeration, which would be helpful for promoting root respiratory metabolism, thus affecting the activities of key enzymes of nitrogen metabolism.

GS, GOT and GPT are key enzymes of ammonia assimilation and amino acid formation in higher plants, where GS is the center of the multifunctional enzyme, and improving the activity can promote plant ammonium assimilation and nitrogen translocation (Becker et al, 2000; Wang et al, 2003). GOT and GPT, the two metabolism transaminase in plants (Shen and Wang, 1991), can promote amino acid decomposition and synthesis of new amino acids (Wang et al, 2003). Transaminase activity reflects the degree of nitrogen and amino acid metabolisms (protein synthesis and degradation). In this study, aeration improved the activities of GS, GOT and GPT in rice roots, indicating that aeration at the seedling stage plays a catalytic role at the initial stage of ammonium assimilation and nitrogen translocation, which is consistent with our previous study that seedling oxygen is conducive to aerial parts of rice nitrogen absorption and utilization (Zhao et al, 2011).

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