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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 (NO_3-N) concertration, 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

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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/(m^2·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-pyruvic acid transaminase (GPT) and glutamic acid oxaloacetate 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 weight 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 α-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 γ-glutamyl hydroxamate. One unit of enzyme is defined as the amount of enzyme that catalyzed the synthesis of 1 μmol of γ-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).