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Enhancement of porosity and aerenchyma formation in nitrogen-deficient rice roots

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ABSTRACT

Root aerenchyma provides oxygen from plant shoots to roots. In upland crops, aerenchyma formation is induced mainly by oxygen or nutrient deficiency. Unlike upland crops, rice forms root aerenchyma constitutively and also inductively in response to oxygen deficiency. However, the effects of nitrogen deficiency on aerenchyma formation in rice remain unknown although nitrogen deficiency is common in most of the world's soils. We aimed to clarify the spatiotemporal patterns of aerenchyma formation induced in rice roots by nitrogen deficiency upon establishment of reliable growth conditions. Rice was grown hydroponically to evaluate porosity and aerenchyma formation induced by nitrogen and oxygen deficiency. Reliable growth conditions for nitrogen and oxygen deficiency were successfully established, because seedling root elongation was significantly promoted by nitrogen deficiency and inhibited by oxygen deficiency. Porosity was higher in whole roots grown under nitrogen and oxygen deficiency than in the controls. Root aerenchyma production was induced extensively by nitrogen deficiency but partially by oxygen deficiency. Thus the physiological roles of aerenchyma induced by nitrogen deficiency likely differ from those under oxygen deficiency. It indicates a possibility that inducible aerenchyma formation in nitrogen deficiency might promote adaptation to this deficiency by reducing respiration and remobilizing nitrogen, or both.

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1. Introduction

Soil waterlogging is an environmental stress that causes severe reductions in growth and yield in upland crops such as maize [1], soybean [2–4], and wheat [5,6]. Root aerenchyma is important in waterlogging tolerance, because the aerenchyma plays a central role in providing space to transport gases such as oxygen from shoots to roots in plants [7]. Aerenchyma is categorized into two types on the basis of its process of formation. One is lysigenous aerenchyma, which is formed by cell collapse with cell death, and the other is schizogenous aerenchyma, which is formed by cell separation without cell death [8,9]. Generally, lysigenous aerenchyma is classified into two types, depending on the trigger for its formation [10]: one is constitutive and the other is inducible. Constitutive aerenchyma is formed with root development [11], whereas inducible aerenchyma is formed in response to environmental factors such as waterlogging [12–14], drought [15,16], or nutrient deficiency [17–19].

In many upland crops, the major type of lysigenous aerenchyma is inducible, not constitutive [12,13,15–19]. Inducible aerenchyma formation in roots has been studied well by taking both anatomical and physiological approaches. Anatomical approaches have shown that inducible aerenchyma in maize is formed by waterlogging, mechanical impedance, drought, and nutrient deficiency [16,17,20–22]. Physiological approaches have shown that aerenchyma formation results from stimulation by, for example, ethylene, reactive oxygen species, calcium signaling, and enzymatic cell-wall degradation [19,22,23]. These studies have facilitated our understanding of inducible aerenchyma formation in upland crops in response to multiple environments.

In contrast, wetland plants such as rice can form both constitutive and inducible root aerenchyma [24]. Rice is therefore useful for studying constitutive and inducible aerenchyma formation. However, studies of inducible aerenchyma formation in rice have received less attention than those in upland crops, because in rice roots it is difficult to estimate whether aerenchyma have formed constitutively or inducibly. To solve this problem, we need to establish reliable growth conditions that enable clear evaluation of inducible aerenchyma formation, with a focus on particular environmental factors. Successful establishment of growth conditions has already provided new information on inducible aerenchyma formation in rice roots. For example, inducible aerenchyma formation as a result of waterlogging was evaluated successfully in an agar solution; the study revealed that aerenchyma formation

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was induced by oxygen deficiency [25,26]. Inducible aerenchyma formation in response to increased osmotic pressure has been evaluated after the successful establishment of sandwich methods with mannitol; the study revealed that aerenchyma formation was also induced by osmotic stress mimicking drought stress [27]. However, whether inducible aerenchyma forms in response to nitrogen deficiency remains unknown, even though nitrogen is an essential nutrient that is commonly deficient in soils.

Our objectives were (i) to establish growth conditions that would enable us to evaluate inducible aerenchyma formation in response to nitrogen deficiency in rice roots; and (ii) to clarify the spatiotemporal pattern of aerenchyma formation induced by nitrogen deficiency.

2. Materials and methods

2.1. Growth conditions in hydroponic culture

Rice plants (*Oryza sativa* L. 'Koshihikari') were hydroponically grown in a growth chamber (LH-220S, NK Systems, Tokyo, Japan) controlled at 28 °C with a light cycle of 14 h light (200–250 μmol quanta $m^{-2} s^{-1}$) and 10 h darkness. Well-filled seeds were preselected by sedimentation in a salt solution of specific gravity 1.12 and then imbibed as described previously [28]. The components of basic nutrient solutions (pH 5.5) (minus nitrogen) was based on the previous work [28] and Mae's solution [29] with slight modifications such as: (1) 5 mM 2-(*N*-morpholino) ethanesulfonic acid (MES) was used to maintain the pH of the nutrient solutions and (2) the nutrient solutions were renewed every two days after sowing until the final day. Plants were grown in established and previous conditions to verify the benefits of the modifications on environmental factors, i.e. pH, dissolved oxygen, and redox potential (Eh). A total of 66 germinated seeds were sown on a net floated with the aid of polystyrene in a pot (275 mm wide × 131 mm deep × 267 mm height) at 2 days after seed imbibition in a Petri dish. Seeds were sown under three conditions. First, plants were grown for 10 days in nutrient solution supplied with 250 μM NH_4Cl and 8.2 mg O_2 L^{-1} by bubbling air for 1 h (control conditions, +N + O_2). Second, plants were grown for 10 days in nutrient solution without nitrogen but with 8.2 mg O_2 L^{-1} by bubbling air for 1 h (nitrogen-deficient conditions, –N + O_2). Third, plants were grown for the first 2 days in +N + O_2 and then transferred to deoxygenated nutrient solutions supplied with 250 μM NH_4Cl and 0.1% (w/v) agar [30] (oxygen-deficient conditions, +N–O_2). The nutrient solutions were renewed every 2 days after sowing.

2.2. Monitoring of pH, dissolved oxygen concentration and redox potential in nutrient solutions

Dissolved oxygen concentration and Eh in nutrient solutions were monitored at 0, 2, 4, 6, 8 and 10 days after sowing (DAS) to assess their statuses before or after exchange of the solution. The dissolved oxygen concentration was measured with a dissolved oxygen meter (SevenGo pro dissolved oxygen, Mettler Toledo Inc., Greifensee, Switzerland) equipped with a dissolved oxygen probe (InLab 605T oxygen sensor IP67, Mettler Toledo). The Eh in the nutrient solutions was measured 100–150 mm below the surface of the nutrient solutions with an Eh meter (PRN-41, Fujiwara Scientific Co. Ltd., Tokyo, Japan).

2.3. Assessment of growth parameters

Plants were grown for 10 days under the three different conditions, i.e. control conditions, nitrogen-deficient conditions, and oxygen-deficient conditions. Shoot length and maximum root length of seedlings were measured with a ruler every 2 days to assess phenotypic responses to nitrogen and oxygen deficiency. On the final day, plants were harvested for measurement of shoot length and maximum root length. After measurement of these lengths, the plants were divided into two portions, shoot and roots, and then dried for at least 7 days at 60 °C. The shoot and roots were weighed with an electric balance (MS104S, Mettler Toledo).

2.4. Measurement of porosity

Whole roots of seedlings grown for 10 days under the three different conditions were harvested for measurement of porosity. Porosity was measured by a pycnometric method [31], with minor modifications. Briefly, a MM-300 mixer mill (Retsch, Haan, Germany) was used for homogenization. Fifty-milliliter pycnometers pre-warmed by submergence in a water bath maintained at 25 °C were used for the measurement.

2.5. Evaluation of aerenchyma formation in cross-sections of seminal roots

Cross-sections were prepared to evaluate aerenchyma formation in the seminal roots in two different experiments. First, to evaluate spatial aerenchyma formation along the root axis, roots were consecutively cut into 5-mm-long segments from a distance of 10 mm behind the root tip to a distance of 10 mm below the basal region (i.e. the root–shoot junction). Second, to evaluate spatiotemporal aerenchyma formation, roots were cut into 5-mm-long segments in four regions, namely from 10 to 15 mm and 20 to 25 mm behind the root tip; in the middle portion of the root; and from 10 to 15 mm below the root–shoot junction. The segments were manually cut into cross-sections with double-edge razor blades (Hi Stainless, Feather Safety Razor Co., Ltd., Osaka, Japan). Subsequently, the cross-sections were cleared (for capture of fine images and fast microscopic observation) with a clearing solution in accordance with the method [32], with three minor modifications as follows: the cross-sections were immersed in a test tube containing clearing solution (85–92% lactic acid saturated with chloral hydrate) (1), incubated overnight at 60 °C (2), and then kept at room temperature until microscopic observation (3). Cross-sections were mounted on slide glass and viewed with bright-field illumination under a microscope (Nikon Microphoto FXA; Nikon Co., Tokyo, Japan) fitted with a CCD camera (camera head, DS-Fi1, Nikon; camera control unit, DS-L2, Nikon). The portions of aerenchyma were delineated by tracing their borders with a pen tablet (Intuos 4, Wacom Co., Ltd., Saitama, Japan), and the area of aerenchyma were calculated with Image J ver. 1.39u software (National Institutes of Health, Bethesda, MD, USA).

2.6. Statistical analyses

All data are represented as means ± standard error (s.e.). One-way analysis of variance with Tukey's Significant Difference procedure was performed by using software (Social Survey Research Information Co., Ltd., Tokyo, Japan).

3. Results

3.1. Verification of the environmental factors using nutrient solutions established in this study and previous study

To verify the effects of the modifications, plants were grown under three conditions: (1) control, (2) nitrogen deficient, and (3) oxygen deficient using the nutrient solutions in established and previous studies. The nutrient solution for the established condition was renewed and pH was adjusted using the MES buffer every two days. Three environmental factors, i.e. pH, dissolved oxygen, and Eh were measured every 2 days, in two conditions before or after

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