Enhancement of porosity and aerenchyma formation by nitrogen deficiency in rice roots (*Oryza sativa* L.)

Lysigenous aerenchyma is formed by cell collapse accompanied with cell death. The aerenchyma in roots provides oxygen from the ground portion to the roots, and is concerned with waterlogging tolerance in plants. Field crops such as wheat show generally poor tolerance to water logging, whereas semiaquatic crops such as rice show high tolerance to water logging. One of the major reasons for the difference in tolerance levels is the initiation of aerenchyma formation between these crops. In wheat, aerenchyma is inducibly formed by multiple environmental factors such as oxygen and nutrient (N, P, K) deficiencies. On the other hand, rice forms two kinds of aerenchyma: constitutive and inducible aerenchyma. Thus, the mechanism of aerenchyma formation in rice is more complicated compared with field crops. Moreover, the mechanism of aerenchyma formation induced by nitrogen deficiency has remained unknown, although the resulting aerenchyma is likely to reduce energy loss. In this study, we attempted to (1) establish reliable growth conditions to estimate aerenchyma formation, and (2) reveal the pattern of aerenchyma formation induced by nitrogen deficiency in rice roots.

Before evaluating aerenchyma formation, we modified the growth conditions, e.g., hydroponic solution and growth period, to estimate precisely the response by nitrogen deficiency alone. We could then establish precise growth conditions demonstrating the recovery of growth vigor caused by pH reduction in hydroponic solutions (Fig. 1). Compared with nitrogen sufficiency, nitrogen deficiency facilitated the formation of air space in whole roots, i.e., an increase in porosity (Fig. 2). In order to determine the spatial and temporal patterns of aerenchyma formation induced by nitrogen deficiency, cross-sections from seminal roots of seedlings grown only on nitrogen-deficient and oxygen-deficient conditions were prepared at several positions, from the root tip to the root base. Microscope observations revealed that aerenchyma formation was enhanced in both nitrogen- and oxygen- deficient conditions compared with reference condition (Fig. 3). In nitrogen-deficient conditions, aerenchyma formation initiated close to root base. Conversely, in oxygen-deficient conditions, the initiation was observed close to root tip (Fig. 3).

As far as we know, this is the first evidence that nitrogen deficiency in rice roots enhances porosity and aerenchyma formation. It strongly distinguishes the physiological roles of nitrogen deficiency and oxygen deficiency on induced aerenchyma formation, demonstrating the different initiation patterns of aerenchyma between nitrogen and oxygen deficiency. Aerenchyma induced by nitrogen deficiency may function in reducing respiration and remobilization of nitrogen, or both. Furthermore, our established growth condition is expected to isolate causal genes associated with aerenchyma (formed either constitutively or induced) toward developing molecular breeding techniques for conferring waterlogging tolerance in field crops in the near future.

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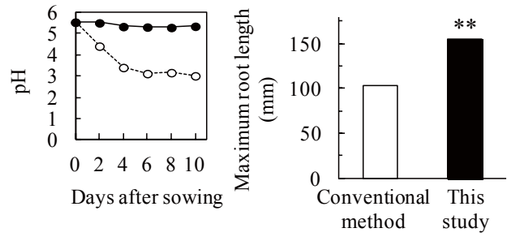

Fig.1. pH maintenance of nutrient solutions (left) and recovery of root elongation (right) in this study (improved method). ○ : conventional method, ●: this study
Asterisks (**) represent a significant difference in maximum root length between conventional method and improved method at *P*-value of 1% level (one-way ANOVA).

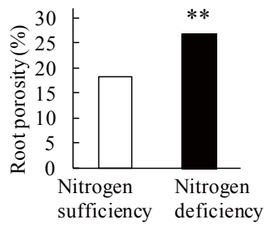

Fig. 2. Increased root porosity by nitrogen deficiency.
Root porosity refers to the size of air space including aerenchyma. Ten-day-old seedlings were used. Asterisks (**) represent a significant difference in root porosity between nitrogen-sufficient conditions and nitrogen-deficient conditions at P-value of 1% level (one-way ANOVA).

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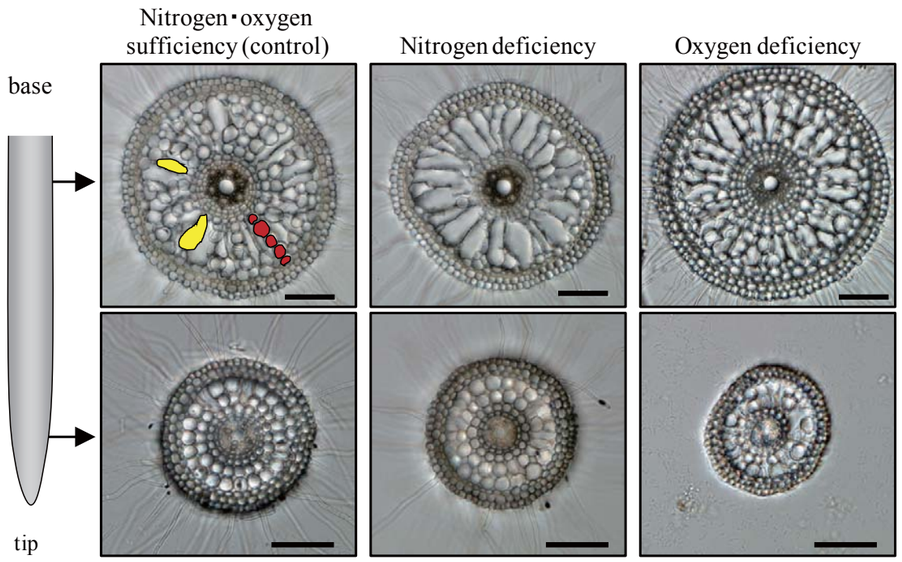

Fig. 3. Root aerenchyma in rice.
Increased aerenchyma was formed by nitrogen deficiency or oxygen deficiency. Examples of cortical cell, which are living cells, were illustrated in red. Examples of aerenchyma, which are dead cells, were illustrated in yellow. Scale bar in individual pictures indicates 100 μm.