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Nodes protect against drought stress in rice (*Oryza sativa*) by mediating hydraulic conductance

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ABSTRACT

The importance of rice resistance to abiotic stress has largely been attributed to signal-induced closure of stomata, protection of antioxidase enzymes, and osmotic adjustment of sugars, etc. However, regulation role of nodes in water flow under drought stress has not been reported. Two rice genotypes Z4 and its recombinant inbred line (RIL) were subjected to drought stress for 10 d at the pollen mother cell meiosis stage. Results indicated a higher decline in spikelet fertility was found in RIL than Z4 plants under drought stress compared with the corresponding control. However, the leaves of Z4 plants were wilting 1 d earlier than those of RIL plants under drought stress. Accordingly, a greater decline in the RWC and water potential of the leaf and sheath, but a lower decrease in the panicle, was observed in the Z4 plants than the RIL plants. In the process, higher decrease in transpiration rate of leaf, hydraulic conductance, diameter of the xylem vessel and relative expression level of *aquaporins (AQP)* genes of node were observed in Z4 than RIL plants under drought stress compared with the corresponding control. Thereby, we deduced that the node act as a "node switch" to control panicle water status by mediating the hydraulic resistance through changing the xylem diameter and the expression level of *AQP* genes under drought stress.

1. Introduction

Plants are constantly challenged by a variety of environmental stresses. One of these is drought, which constitutes a crucial limiting factor for plant growth and is expected to become increasingly prevalent in many regions as a result of climate change (Bartels and Sunkar, 2005; Diffenbaugh et al., 2015). Rice (*Oryza sativa*) is sensitive to drought stress and experiences significantly reduced grain yield when drought stress occurs at the reproductive stage, which, in extreme cases, can lead to no harvest (Fu et al., 2011). In response, rice plants have evolved a range of drought stress strategies. And one of these is to decrease water conductance along the soil-plant-atmosphere water pathway in order to reduce transpiration (Maurel et al., 2016; Sperry et al., 2016).

Drought resistance is determined by the ability of a plant to maintain water balance, including water uptake and water loss under drought conditions, during which stomatal resistance constitutes a key component in resisting water flow (Garcia-Forner et al., 2016). However, this process is also mediated by the hydraulic conductance of the roots and shoots, which is decreased under drought stress due to the interruption of the water column in the vessels or modifications to the size of the xylem conduits (Jansen et al., 2011). Under drought stress, the decrease in the hydraulic conductance of the roots impairs drought tolerance as a result of higher water uptake resistance (Alsina et al., 2011; Domec et al., 2010). In contrast, reduced shoot hydraulic conductance is a water-saving strategy used by rice plants under drought conditions, as it reduces water flow across the shoots and induces stomatal closure, which deters further embolisms and limits transpiration (Singh et al., 2010, 2011). Among the shoot segments, the greatest hydraulic resistance under drought stress is found in the stem, followed by the leaf sheath and lamina (Singh et al., 2010). However, an aspect that has seldom been reported on is the protective function of nodal xylem conductance under drought stress.

In general, a rice culm contains 13 to 16 nodes, a junction of vasculatures connecting the leaves, stems, and panicles. Node I beneath the panicles has large and small vascular bundles and diffuse vascular

Abbreviations: AQP, aquaporin; CC, companion cells; DADS, days after drought stress; DR, decease range; DS, drought stress; PPC, phloem parenchyma cells; PCB, parenchyma cell bridge; RIL, recombinant inbred line; RWC, relative water content; VB, vascular bundle; XTC, xylem transfer cells

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bundles, which originate from the lower nodes and connect to the flag leaf and are markedly enlarged at the node (Yamaji and Ma, 2014). It has been well-documented that the nodes play an important role in mineral nutrient distribution, and four modes of distribution based on specific molecular transport processes for different mineral elements, including xylem-switch, phloem-tropic, phloem-kickback, and minimum-shift, have primarily been identified in the nodes of rice (Yamaji and Ma, 2009, 2014; Yamaji et al., 2013). However, the role of the nodes in water flow in rice plants under drought stress has not previously been documented. Thus, in this study, the relative water content (RWC), water potential, transpiration rate, hydraulic conductance, xylem morphology and expression level of *aquaporins* (*AQP*) genes were assessed in an attempt to reveal the mechanisms underlying the function of the nodes in drought stress survival in rice.

2. Materials and methods

2.1. Plant materials and drought stress treatments

Experiments were performed at the Fuyang farm of the China National Rice Research Institute, Hangzhou, Zhejiang Province, China. Two rice genotypes, namely Z4 (higher drought resistance) and its recombinant inbred line (RIL, lower drought resistance), were used in this experiment. After soaking and sprouting, the seeds were directly sowed into pots with the height and diameter of 30 cm, and then thinned to four seedlings per pot after 30 days. Each pot was filled with 15 kg of paddy soil blended with 20 g of compound fertilizer (N: P: K = 14:16:15). The rice plants were grown in a large temperature-controlled chamber under natural sunlight conditions, in which the temperature and relative humidity were controlled at 30/24 °C and 70/80% (day/night), respectively. Drought stress treatments were imposed at the meiosis stage (length between pulvinus of flag leaf and the second leaf was approximately −3 cm) by withholding water for 10 d. Totally 40 pots were prepared for each rice genotype, in which 18 pots with plants were under control while 22 pots with plants were under drought stress.

2.2. Determination of RWC and water potential

Three pots with plants were mainly used to collect the soil samples with root residues in the middle of the pot from 10 to 15 cm depth at 2, 4, 6, 8, and 10 days after drought stress (DADS) and dried in an oven at 105 °C to constant weight. The soil contained root residues weight before and after drying was recorded, and the water content was determined by calculating the difference. Another six pots (three pots under control while another three pots under drought stress) with plants were used to determine the RWC of panicles, sheaths, and leaves at 9 DADS. The rice plants were divided into panicles, flag leaves, sheaths and weighed (*Wf*) immediately. They were then immersed in deionized water for 24 h and the saturated weight was recorded (*Ws*). Following this, they were dried at 75 °C to constant weight and the dry weight (*Wd*) was determined. RWC was calculated as (*Wf-Wd*)/(*Ws-Wd*) × 100% (Schonfeld et al., 1988).

A PSYPRO Dew Point Microvoltmeter equipped with the psychrometer sensor (Model PST-55, Wescor Company, USA) was used to determine the water potential of the soil at 2, 4, 6, 8, and 10 DADS. The soil hygrometer was deeply imbedded in 10 cm under the topsoil. The record was taken at 8:00, 12:00 am and 4:00 pm for three times and calculated as the average within one determination day. The water potential of plant organs including panicle, flag leaf and its sheath were determined at 9 DADS with a leaf sample chamber (Model C-52, Wescor Company, USA). Discs of plant samples obtained by using a piercer, were placed inside the measuring chamber. After a thirty minutes' equilibrium of the microenvironment in measurement chamber, the readings were taken by the system. For both determinations, three replicates were conducted in each treatment.

2.3. Root bleeding assay

For the determination of root bleeding rate, ten stems from different single plants with uniform growth status were chosen for each treatment. Incisions were made approximately 10 cm above the base of the plants at 6:00 (p.m.) of 9 DADS. The wounds were then wrapped in cotton balls and sealed with preservative film. The absorbed quantity of root bleeding exudate was collected after 12 h and weighed by subtracting the cotton weight from the total weight. The intensity of the bleeding was expressed as the bleeding quantity in the culms of each plant per hour (Li et al., 2012).

2.4. Photosynthesis measurement

The net photosynthetic rate (P_N), stomatal conductance (Cond), and transpiration rate (Tr) of the flag leaves were determined at 0, 8, and 9 DADS during 9:00–11:00 (a.m.) using a Li-COR 6400 portable photosynthesis system (Li-COR Biosciences Inc., Lincoln, NE, USA) under the following conditions: photosynthetic photon flux density of 1200 μmol m^{-2} s^{-1}, ambient CO_2 concentration of 400 μmol mol^{-1}, 500 μmol s^{-1} for flow speed and at the temperature of 30°C. Six leaves that had their upper surfaces exposed to the sunlight were selected and measured.

2.5. Determination of nodal hydraulic conductivity

The hydraulic conductivity of node was measured as described by Wang et al. (2013) with modifications. The whole main stems of three rice plants were picked and taken as samples. The panicle, leaf and sheath were immediately removed from the stems. The uppermost node with approximately 1 cm at both ends of stem was positioned horizontally. One side was connected to a flexible rubber hose and the other side connected to a pipette, both of which were swathed to refrain from possible leaking. The hose was immersed in deionized water in a graduated cylinder, and the middle of the hose was equipped with an injector to eliminate air. A perpendicular distance of 1 m was required between the cylinder liquid level and the stem. There was some water flow in the detached stem due to the hydraulic pressure produced by the water column. The flow speed was calculated by dividing the volume and the time taken to pass through the system and was expressed by F (kg s^{-1}). Conductance was deduced by using the formula $F{\cdot}L/\triangle P$, where, L is the length of the stem and $\triangle P$ is the pressure intensity that induced the flow.

2.6. Xylem morphology observation

Node segment samples were harvested at 9 DADS and fixed in 2.5% cold glutaraldehyde solution, and then preserved in 70% ethanol for microscopy. The position at which the node was taken for this observation was in accorded with that for hydraulic conductivity determination. Sections were made using a sliding microtome (Leica SM2010, Germany). Transections of the nodes were stained with 1% safranine for 1–2 h and 0.5% fast green for 30–60 s before decolorizing with anhydrous ethanol and placement on a microscope slide. Concurrently, 1% aniline blue was used for xylem staining. Tissue slices were photographed with a florescence microscope (Leica DM4000, Germany). At least twenty photographs displaying the intact tissues for each replicate treatment were taken at 200-fold and 400-fold eyepiece. The total numbers of vessel bundles, xylem vessels and their dimensions were measured using Image J software (US National Institutes of Health, Bethesda, MD, USA) by selecting the calculation tool for irregular delineation.

2.7. Gene expression determination of AQP

Three *AQP* genes, including *PIP1;1*, *PIP2;4*, and *PIP2;7* were