Morphological and histological differences among three types of component roots and their differential contribution to water uptake in the rice root system

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\begin{abstract}
The rice root system consists of three types of roots; main root, L-type and S-type lateral root (LR). These component roots are morphologically and histologically different, which is termed as heterorhizy. Root system hydraulic architecture is related to the unique features of component roots. We hypothesized that each component root contributes in different degrees to water uptake of the whole root system. Rice varieties IRAT 109 and Taichung 65 were grown in pots filled with soil under continuous waterlogged (CWL) and drought (CD) conditions until two weeks after heading. Morphology and histological structures of roots, which may regulate radial water movement, were compared among the three component roots. Moreover, hydraulic conductivity ($L_p$) of the root system, which represents the water uptake ability, were measured with a pressure chamber. Based on a model that $L_p$ of the whole root system is a product of $L_p$ of each of the component roots and their surface areas, we found that the differences in $L_p$ between the two varieties and the plants grown under different soil water conditions for any of the component roots did not support the corresponding differences in the measured $L_p$ of the whole root system. In contrast, a significant and positive correlation was found between $L_p$ of the whole root system and the percentage of surface area of S-type LR but not for the other component roots. These results indicate S-type LR might have a higher contribution to $L_p$ of the whole root system than the other component roots.
\end{abstract}

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\section*{Introduction}
The rice root system consists of a number of component roots; main roots including one seminal root and nodal roots, and two types of lateral roots (LRs) (L-type LR and S-type LR) (Kono et al., 1972; Yamauchi et al., 1996). L-type LR is generally thick and long while S-type LR is mostly thin and short. Additionally, the L-type LR has the
ability to branch into higher order L- and S-type LR, but the S-type LR does not have such ability (Kono et al., 1972). Furthermore, there are gradients in root age acropetally from root base to root apex along each individual root axis, and such age distribution can apply to every LR (Hishi, 2007). Thus, the root system of an individual plant is composed of different roots with various morphology and ages, which is termed ‘heterorhizy’ (Kumazawa, 1979).

Among the root types, more than 90% was accounted for by LRs in total root length, indicating the greater contribution of LRs to water uptake (Doussan et al., 1998; Varney & Canny, 1993; Yamauchi et al., 1996). Muller et al. (2019) recently pointed out the functional significance of random diversity in LRs. Gu et al. (2017) also classified LRs based on their diameter and length, and discussed their possible importance in water and nitrogen uptake by examining their developmental responses to different conditions of soil water and nitrogen application rates. Our studies also showed that L-type LR production and elongation were promoted in response to mild drought stress, which implied the differences in responses to environmental conditions between L-type LR and S-type LR (Banoc et al., 2000; Kano et al., 2011; Suralta et al., 2010, 2018). Such developmental response is known as root plasticity and is one of the key traits of plant adaption to various abiotic stresses (Suralta et al., 2018).

The unique features of the component roots are assumed to closely affect the hydraulic architecture of the root system. It is well established that the hydraulic architecture of a root system is determined by the hydraulic conductance \( L_r \) which represents water uptake and transport ability. \( L_r \) has been reported to be regulated mainly by root length/surface area in many cases (Gowda et al., 2011; Suralta et al., 2018). On the other hand, the water flow per unit root surface area (RSA) is defined as hydraulic conductivity \( L_{P_r} \) which is likely affected by root anatomical structure such as number of cell layers (Bramley et al., 2007; Rieger & Litvin, 1999), the degree of cell disintegration in the cortex (Hayashi et al., 2011; Yang et al., 2012), and cell wall modification such as the development of Casparian bands as the primary wall, deposition of suberin lamellae as the second wall, and the formation of tertiary walls (Ma & Peterson, 2003; Schreiber, 2010) as well as the physiological activity of aquaporin genes (Maurel et al., 2008). Number of cell layers from root surface to the xylem vessel, aerenchyma formation and deposition of suberin and lignin which are the biopolymers contained in Casparian bands, suberin lamellae and tertiary walls may decrease \( L_{P_r} \) in the radial direction.

In contrast, enhanced aquaporin activity may contribute to increased water permeability of cell membranes (Moshelion et al., 2009). Among component roots, in addition to morphological and developmental features, differences in inner histological structures such as endodermis, xylem dimensions have also been reported, which may also affect \( L_{P_r} \) (Henry et al., 2016). These facts strongly lead to the assumption that each component root may have different \( L_{P_r} \). Therefore, the functional roles of these component roots in water uptake may be different from each other although no studies have focused on the heterorhizy from the aspect of root hydraulics.

It has been proposed in a model that the water flow and \( L_r \) of the whole root system are the sum of these of roots composing the root system (Adachi et al., 2017; Knipfer & Fricke, 2011; Suku et al., 2014), and therefore, \( L_{P_r} \) of the whole root system can be related with \( L_{P_r} \) of each component root and their RSA as expressed by the following the equation;

\[
L_{P_r}(\text{whole root system}) = \sum \left[ L_{P_r}(\text{each component root}) \right. \\
\times \left. \left( \frac{\text{RSA(each component root)}}{\text{RSA(whole root system)}} \right) \right]
\]

where the \( L_{P_r} \) of the whole root system may be determined either by \( L_{P_r} \) of each component root or the composition of different component roots, or both, which may also change in response to soil water condition and other abiotic stresses. As such, investigation of functional roles of each component in water uptake should significantly contribute to our understanding of the mechanism of water uptake by the whole root system, which is crucial to design the ideology of root system adapted to water stress conditions that mainly limit rice production worldwide (Kano-Nakata et al., 2019).

Further, water flows in each component root, which is driven by two different forces (hydrostatic and osmotic potential gradients), pass via two different pathways including the apoplastic and cell-to-cell pathway (Boyer, 1985). The water flow driven by hydrostatic potential gradient is dominant when the plant is at high transpiration rate and in this situation, water mainly flows in apoplastic pathway. On the other hand, the contribution of the water flow driven by osmotic potential gradient is relatively high when plants are at low transpiration rate, and cell-to-cell pathway is more dominant than apoplastic for water flow (Steudle & Peterson, 1998).

The objectives of this study, therefore, were (1) to identify morphological and histological differences among the component roots which may closely regulate \( L_{P_r} \), (2) to quantitatively evaluate the contribution of \( L_{P_r} \) and the composition of each component root to \( L_{P_r} \), of
the whole root system of plants grown under different soil water conditions, and (3) to compare hydrostatic hydraulic conductivity ($Lp_r$ (hydrostatic)) and osmotic hydraulic conductivity ($Lp_o$ (osmotic)) in each component root. Our hypothesis for the first objective was that histological structures and morphological characteristics, which are closely related with $Lp_r$, would differ among the component roots. The second hypothesis was that changes in $Lp_r$ and in the proportions of component roots in a whole root system of plants when grown under different soil water conditions might regulate $Lp_o$ of the whole root system. The third hypothesis was that the dominant water pathway may be different among the component roots with the expectation that more water flows through cell-to-cell pathway than apoplastic pathway in S-type LR which may have less hydraulic resistance due to less number of cell layers and no aerenchyma formation, as compared with L-type LR and main root. Overall, this study aimed to examine the differential functional roles of the three component roots in the hydraulic architecture of the rice root system.

**Materials and methods**

**Plant materials**

Japonica varieties, IRAT 109 and Taichung 65 were used in this study. In previous studies, we found that IRAT 109, an upland variety, promoted LR development in response to rewatering after drought treatment (Bañoc et al., 2000) and showed greater ability to increase root length density especially in the deeper soil layer (Kameoka et al., 2015). Thus, IRAT 109 may show different morphological and developmental responses to soil water conditions as compared with Taichung 65, a lowland variety which showed significant decrease in $Lp_r$ under drought condition in a preliminary experiment.

Seeds of each variety were soaked in water containing benomyl fungicide (benlate, Sumitomo chemical garden products Inc. Tokyo, Japan, 0.15%, w/v) and then incubated at 30°C for 24 hours. After 24 hours, benlate was replaced with tap water and the seeds were kept in an incubator at 30°C for another 48 hours. One germinated seed was sown in a plastic pot ($\phi$175 × $\phi$160 × 198 mm, upper diameter × lower diameter × height) filled with 4.0 kg of air-dried sandy loam soil on the 3 August 2017. Chemical fertilizer (N:P:K = 14:14:14, Itochu Co. Tokyo, Japan, 0.64 g pot$^{-1}$) was top dressed on the 10th August and the 22 September 2017. Pots were arranged in a randomized complete block design with three replicates and placed in a greenhouse at Nagoya University, Japan and the plants were grown until two weeks after heading.

**Soil water treatment**

By using the two varieties that are expected to show contrasting developmental responses to soil water conditions, the plants were exposed to two different soil water conditions; continuous waterlogged (CWL), or continuous drought (CD). Soil water content (%, w/w) was regularly checked by weighting the pots, and calculated to soil water potential (KPa, SWP) following the pre-determined equation of soil water content that was calculated with the weight of pot vs soil water potential which was measured by using a soil tensiometer (Daiki soil and moisture, Daiki Rika Kogyo Co., Japan). In the CWL control, soil water content was maintained over 25% (0 KPa in SWP) throughout the experiment. In the CD treatment, soil water content was maintained to 15.3% (~36 KPa in SWP) on average throughout the experiment except for the first 35 days when the soil was first saturated with water at sowing.

**Measurements**

**Root diameter, aerenchyma formation, and suberin lamellae and lignin deposition in each component root**

The morphological features and inner histological structure were compared among component roots of plants grown under CWL and CD conditions. Three main roots, which were longer than the other main roots, with developed LRs at apical portions, were sampled from each root system. Main roots were excised into 1-cm segments at 25%, 50% and 75% relative position in length from the root base. L-type LR and S-type LR branched from each main root segment were also excised. For observations and measurements, cross sections of each component root were made with the thickness of 100 μm for main root, 60 μm for L-type LR and S-type LR using a microtome (VT1200 S, vibrating blade microtome, Leica, Germany).

The root diameter of each component root, and aerenchyma area of the main root and L-type LR were measured by using Image J software. Aerenchyma area (%) was calculated according to the following equation (Visser & Bögemann, 2003).

$$\frac{\text{Aerenchyma area (μm}^2\text{)}}{\text{total cross section area (μm}^2\text{)}} \times 100$$

To detect the deposition of aliphatic suberin, which has stronger function as the apoplastic barrier than aromatic suberin (Schreiber et al., 2005; Schreiber et al., 1999), cross sections were stained for 1 hour with Fluorol Yellow 088 (Ranathunge et al., 2011) at room temperature. Cross
sections were viewed under an epifluorescence microscope using an ultraviolet filter set (excitation filter BP 330–385, dichroic mirror FT 400, barrier filter LP 420; Model: IX70, inverted fluorescence microscope, Olympus, Tokyo, Japan) and aliphatic suberin can be detected with yellow fluorescence under ultraviolet light. The digitized images were taken at the same time by using CCD camera (DP21, Olympus, Tokyo, Japan) together with non-stained samples. To observe the deposition of lignin that is a component of tertiary wall, cross sections were stained for 10 min with phloroglucinol (final concentration; 1% (w/v)) which was mixed with 75% ethanol at room temperature and then 6 N HCl was dropped on the samples (Wiesner reaction, Davidson et al., 1995). Sections were viewed under a light microscope (IX70, inverted fluorescence microscope, Olympus, Tokyo, Japan) and the digitized images were taken at the same time by using a CCD camera (DP21, Olympus, Tokyo, Japan) together with non-stained samples.

**Hydrostatic hydraulic conductance (Lr, (hydrostatic))/conductivity (Lp, (hydrostatic)), osmotic hydraulic conductance (Lr, (osmotic))/conductivity (Lp, (osmotic)), and root surface area (RSA)**

Root hydrostatic hydraulic conductance (Lr, (hydrostatic)) and root hydrostatic hydraulic conductivity (Lp, (hydrostatic)) of plants grown under CWL and CD were measured using a pressure chamber (φ267.4 × 778 mm, diameter × height) which was constructed at the Extreme Environments Equipment Development Group of Equipment Development Support Section, Technical Center, Nagoya University. Before the measurement, non-productive tillers and dead leaves were removed, and then productive tillers with panicles were completely sealed and installed to the PVC pipe joint with silicon sealant up to 10 cm from the base to prevent air leakage between the plant and the chamber during pressure application. Pots were left in a well-ventilated place to dry the silicon sealant for 36 hours.

The measurement was conducted in an experimental room, where the room temperature was kept at 25.1 ± 0.6°C (±SD) on the 5 December 2017 (94 DAS) for IRAT 109, and the 9 December 2017 (98 DAS) for Taichung 65. After checking the sealant was dried, each pot was waterlogged to fill the gap between soil and root surface, and thus to minimize the hydraulic resistance at the soil-root interface, and stably placed in a chamber and the pipe joint was firmly fixed with two types of disk-like lids. Stems were cut with scissors at 13 cm from the base, and exudation rate was measured every 60 s under non-pressure application condition (0 MPa) by weighting cotton which was put on cut-end of stems and covered with plastic film to prevent evaporation as described by Matsuo et al. (2009). After measuring exudation rate at 0 MPa, 0.10 MPa pressure was applied by using an air compressor (ACP-25SLA, Takagi Co., Ltd, Japan) for 10 min in idle running mode to prevent cavitation inside the roots. Then, 0.10, 0.08, 0.06, and 0.04 MPa pressure was applied for 180 s (60 s × 3 times) each and the cotton was weighted every 60 s. Lp was calculated based on the slope of a linear relationship of xylem sap exudation rate at each pressure.

After the measurement, the root samples were washed carefully to remove the soil, and total RSA was measured by scanning the roots at 600 dpi using an EPSON scanner, which was then analyzed using the root analysis software WinRHIZO 2016 (Regent Instruments Inc., Saint-Foy, Canada). The roots were grouped into three types of component roots; main root, L-type LR and S-type LR according to the diameter estimated with WinRHIZO 2016 (Table 1) based on the preliminary measurement of actual diameter of L-type and S-type LRs with image J software for 10 randomly-chosen roots each. L-type and S-type LRs were classified first visually according to the existence of branched roots, and then confirmed anatomically with aerenchyma formation and early metaxylem vessels which S-type LR does not have whereas L-type LR does. Then the surface area was determined for each component root. Xylem sap which was collected with cotton, was squeezed and used for the osmotic potential measurement using a vapor pressure osmometer (model 5520, Wescor Inc., USA). Finally, using these data, the following four root hydraulic parameters were calculated:

\[ Lr(\text{hydrostatic}) \left( \text{m}^3\text{s}^{-1}\text{MPa}^{-1} \right) = \frac{Q}{\Delta\psi} \]

where Q is the exudation rate (m³ s⁻¹), Δψ is the driving force (MPa) which is the sum of applied pressure in a chamber and the difference in osmotic potential between the medium and xylem sap.

\[ Lp_r(\text{hydrostatic}) = \frac{Q}{(\Delta\psi \times \text{RSA})} \]

\[ Lr(\text{osmotic}) \left( \text{m}^3\text{s}^{-1}\text{MPa}^{-1} \right) = \frac{Q}{\Delta\psi} \]

| Table 1. The diameter (mm) set to each component root of IRAT 109 (94-day-old) and Taichung 65 (98-day-old) for root analysis with WinRHIZO. |
|-------------|-----------------|-----------------|-----------------|
| Variety     | Type of component root | S-type LR (mm) | L-type LR (mm) | Main root (mm) |
| IRAT 109    | L-type LR, L-type lateral root; S-type LR, S-type lateral root. | 0.02−0.14 | 0.14−0.36 | 0.36−0.39 |
| Taichung 65 | L-type LR, L-type lateral root; S-type LR, S-type lateral root. | 0.02−0.10 | 0.10−0.30 | 0.30−0.39 |
where \( Q \) is the exudation rate \( (m^3 \text{s}^{-1}) \), \( \Delta \psi \) is the driving force (MPa) which is the difference in osmotic potential between the medium and xylem sap.

\[
L_p/\text{osmotic}(m^3 \text{m}^{-2} \text{s}^{-1} \text{MPa}^{-1}) = Q/(\Delta \psi \times RSA)
\]
(Meng et al., 2016).

**Results**

*Differences in morphology and inner histological structure among the three component roots of two varieties grown under CWL and CD conditions*

Three types of component roots were compared in their morphology and histological features for the plants grown under CWL condition. For IRAT 109, the differences were recognized in their diameter, branching pattern, the formation of aerenchyma and inner structure of cell layers (Figure 1(a–d)). Such differences were observed also in Taichung 65 (data not shown).

Table 2 shows the root diameter and aerenchyma area (%) that are related with root hydraulic of the two varieties grown under CWL control and CD conditions. Overall, the average diameter of each component root of IRAT 109 was greater than that of Taichung 65 while there were no significant changes with CD treatment in both varieties except for main root of IRAT 109, which showed significant decrease in the average diameter by CD treatment.

The aerenchyma area ranged from 69 to 76 (%) in main roots in both varieties and treatments. In L-type LR, aerenchyma area ranged from 29 to 46 (%), and the aerenchyma area in main root and L-type LR tended to be decreased by CD treatment only in IRAT 109 (Table 2).

The cross sections of each component root were stained for the observation of suberin lamellae of IRAT 109 and Taichung 65 grown under CWL control (Figure 2 for IRAT 109 and S4 for Taichung 65) and CD condition (Figures S3 and S5 in Supplementary Material). Suberin lamellae was observed in the exodermis and endodermis but not in the sclerenchyma in the main root for both varieties grown under CWL as well as CD conditions. In L-type LR, both varieties showed brighter fluorescence yellow color in the endodermis than in exodermis. In S-type LR, suberin lamellae were observed in the endodermis and outer cortex. However, suberin lamellae was not observed in any of the cells in the endodermis unlike the main root and L-type LR in which suberin lamellae was recognized in almost all endodermal cells.

![Figure 1](image-url).

*Figure 1.* Three types of component root in the root system (a), cross sections of the middle portions of main root (b), L-type lateral root (c) and S-type lateral root (d) of IRAT 109 grown under continuous waterlogged (CWL) condition in soil till two weeks after heading observed with a light microscope. L-type LR, L-type lateral root; S-type LR, S-type lateral root. Bars represent 200 \( \mu \text{m} \) (b), 100 \( \mu \text{m} \) (c) and 20 \( \mu \text{m} \) (d). In IRAT 109 (94-day-old) grown under both water conditions, 1\textsuperscript{st} order L-type and S-type LRs were observed to emerge on seminal and nodal root axes and the 2\textsuperscript{nd} order of both L- and S-type LRs were observed on the 1\textsuperscript{st} order L-type LR, while in Taichung 65 (98-day-old) grown under both water conditions, 2\textsuperscript{nd} order LR were observed only for S-type but not L-type.
Lignin deposition of IRAT 109 grown under CWL control and CD condition are shown in Figure 3 and Figure S6 in Supplementary Material. In the main root of IRAT 109, lignin deposited around the cell wall of endodermis and sclerenchyma, and the red color intensity was observed to be stronger than in exodermis. In L-type LR, lignin deposition was also observed in the exodermis and endodermis, although the red color in the exodermis was lighter than in the endodermis. In S-type LR, lignin deposition was observed around the outer cortex and in the outer part of the endodermis but rarely observed in exodermis (hypodermis). No apparent differences were observed in the

| Variety         | Treatment | Type of component root | Main root Diameter (μm) | Aerenchyma area (%) | L-type LR Diameter (μm) | Aerenchyma area (%) | S-type LR Diameter (μm) |
|-----------------|-----------|------------------------|-------------------------|---------------------|------------------------|---------------------|------------------------|
| IRAT 109        | CWL       | Main root              | 1421.0 ± 96.2 c         | 75.9 ± 2.2b         | 249.3 ± 71.5b          | 46.2 ± 8.8b         | 90.4 ± 12.5b           |
| IRAT 109        | CD        | L-type LR              | 1212.0 ± 132.6b         | 70.1 ± 4.4a         | 227.1 ± 39.6ab         | 29.3 ± 5.3a         | 85.7 ± 36.9b           |
| Taichung 65     | CWL       | S-type LR              | 990.4 ± 121.2a          | 68.8 ± 6.9a         | 161.0 ± 38.7a          | 35.8 ± 11.1ab        | 58.3 ± 5.8a            |
| Taichung 65     | CD        |                        | 1110.6 ± 63.4ab         | 73.4 ± 2.3ab        | 202.5 ± 54.3ab         | 32.3 ± 6.0a         | 64.8 ± 14.8ab          |

ANOVA Variety (V) *** ns ns ***
Treatment (T) ns ns ns *** ns
V × T *** ns ns *** ns

Values are shown in Mean ± SD
CWL, continuous waterlogged; CD, continuous drought. L-type LR, L-type lateral root; S-type LR, S-type lateral root.
The values followed by the same letter are not significantly different at P < 0.05 by Tukey’s test.

Figure 2. Cross sections of the middle portions of main root (a and b), L-type lateral root (c) and S-type lateral root (d) of IRAT 109 grown under continuous waterlogged (CWL) condition in soil till two weeks after heading observed with an epifluorescence microscope using an ultraviolet filter set (excitation filter BP 330–385, dichroic mirror FT 400, barrier filter LP 420; Model: IX70, inverted fluorescence microscope, Olympus, Tokyo, Japan). Suberin lamellae stained with Fluorol Yellow 088 are shown in fluorescence yellow color under ultraviolet light. (a), outer part of main root; (b), central cylinder; ep, epidermis; ex, exodermis; sc, sclerenchyma; co, cortical parenchyma; en, endodermis; ae, aerenchyma; lm, late metaxylem. Bars represent 20 μm (a, b, d) and 50 μm (c).
roots grown under CD condition (Figure S6 in Supplementary Material). In addition, similar differences in morphology and inner histological structures among the three component roots were also observed for Taichung 65 (Figures S7 and S8 in Supplementary Material).

Root hydraulic conductance and conductivity, and surface area of two varieties grown under CWL and CD conditions

Root hydraulic conductance was measured with a pressure chamber as water uptake ability of the whole root system. As shown in Table 3, \( L_r \) (hydrostatic) value (±SD) was 111.2 ± 24.1 \((10^{-10} \text{ m}^3 \text{ s}^{-1} \text{ MPa}^{-1})\) for IRAT 109 and 103.9 ± 21.6 \((10^{-10} \text{ m}^3 \text{ s}^{-1} \text{ MPa}^{-1})\) for Taichung 65 in CWL control. In CD treatment, \( L_r \) (hydrostatic) value of IRAT 109 and Taichung 65 was 52.7 ± 15.4 \((10^{-10} \text{ m}^3 \text{ s}^{-1} \text{ MPa}^{-1})\) and 25.2 ± 3.3 \((10^{-10} \text{ m}^3 \text{ s}^{-1} \text{ MPa}^{-1})\), respectively. In both varieties, \( L_r \) (hydrostatic) was significantly decreased by CD treatment, but \( L_r \) (hydrostatic) of IRAT 109 tended to be still higher than that of Taichung 65. \( L_r \) (osmotic) was also higher in IRAT 109 than that of Taichung 65 in both treatments, although the differences among the treatments were not significant in both varieties.

\( L_p \) (hydrostatic) value (±SD) was 100.0 ± 27.1 \((10^{-10} \text{ m}^3 \text{ m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1})\) for IRAT 109 and 103.3 ± 28.3 \((10^{-10} \text{ m}^3 \text{ m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1})\) for Taichung 65 in CWL control (Table 3). Similarly to \( L_r \) (hydrostatic), both varieties showed similar \( L_p \) (hydrostatic) in CWL control. CD treatment did not significantly decrease the \( L_p \) (hydrostatic) of IRAT 109 as compared with CWL control, which tended to be higher than that of Taichung 65. \( L_p \) (osmotic) value (±SD) was 48.8 ± 27.3 \((10^{-10} \text{ m}^3 \text{ m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1})\) for IRAT 109 and 17.0 ± 5.9 \((10^{-10} \text{ m}^3 \text{ m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1})\) for Taichung 65 in CWL control. Similarly to \( L_p \) (hydrostatic), \( L_p \) (osmotic) in CWL control for IRAT 109 tended to be highest among the soil water conditions and varieties. In CD treatment, both varieties showed the tendency of decreased \( L_p \) (osmotic) but there were no significant decrease by CD treatment. \( L_p \) (osmotic) of IRAT 109 again tended to be higher than Taichung 65. Moreover, the \( L_p \) (hydrostatic) values were 2.0–6.1 times higher than \( L_p \) (osmotic) in all the varieties and treatments.

Figure 3. Cross sections of the middle portions of main root (a and b), L-type lateral root (c) and S-type lateral root (d) of IRAT 109 grown under continuous waterlogged (CWL) condition in soil till two weeks after heading observed with a light microscope. Lignin deposition stained with phloroglucinol and HCl are shown in red color. (a), outer part of main root; (b), central cylinder; ep, epidermis; ex, exodermis; hy, hypodermis; sc, sclerenchyma; co, cortical parenchyma; en, endodermis; oc, outer cortex; ae, aerenchyma; lm, late metaxylem; em, early metaxylem; px, protoxylem. Bars represent 20 \( \mu \text{m} \) (a, b, d) and 50 \( \mu \text{m} \) (c).
In CWL control, the whole RSA (±SD) of IRAT 109 and Taichung 65 was 1.13 ± 0.06 (m² plant⁻¹) and 1.03 ± 0.08 (m² plant⁻¹), respectively (Figure 4). CD treatment significantly reduced the RSA in both IRAT 109 and Taichung 65 as compared with the CWL control, and that of IRAT 109 tended to be greater than that of Taichung 65 (Figure 4).

To evaluate the contribution of each component root to the whole root system in terms of surface area, the ratios of each component root to the whole root system were calculated and shown in Figure 5. The percentages of main root and L-type LR showed increased tendency in both varieties in CD treatments as compared to CWL control. In contrast, that of S-type LR was significantly decreased by CD treatments as compared to CWL control in IRAT 109 and Taichung 65. The percentage of surface area of S-type LR to the whole root system (±SD) of IRAT 109 ranged from 34.7 ± 2.3 (%) to 46.6 ± 4.6 (%), and it tended to be higher than those of Taichung 65 in each treatment.

**Correlation between the percentage of surface area of each component root to the whole root system and root hydraulic conductivity**

The contribution of each component root to the whole root system in water uptake was estimated based on the correlation between $L_p$, of the whole root system and the percentage of surface area of each component root to the whole root system. The results showed that there were significant positive correlations between the percentage of surface area of S-type LR, and both $L_p$ (hydrostatic) and $L_p$ (osmotic) of both varieties and treatments (Figures 6 and 7) whereas no such correlation was observed for the main root nor L-type LR (Figures S1 and S2 in Supplementary Material).

### Table 3. $L_r$ and $L_p$, driven by hydrostatic or osmotic potential gradient.

| Variety   | Treatment | $L_r$ (hydrostatic) | $L_p$ (hydrostatic) | $L_r$ (osmotic) | $L_p$ (osmotic) |
|-----------|-----------|---------------------|---------------------|----------------|----------------|
| IRAT 109  | CWL       | 111.2 ± 24.1 c      | 100.0 ± 27.1a       | 53.7 ± 27.1b   | 48.8 ± 27.3a   |
|           | CD        | 52.7 ± 15.4ab       | 63.2 ± 22.7a        | 20.7 ± 4.2ab   | 24.2 ± 4.1a    |
| Taichung 65 | CWL     | 103.9 ± 21.6bc      | 103.3 ± 28.3a       | 17.0 ± 5.0ab   | 17.0 ± 5.9a    |
|           | CD        | 25.2 ± 3.3a         | 38.7 ± 4.6a         | 5.4±±1.7a     | 8.3 ± 2.9a     |

ANOVA Variety (V) ns ns ** Treatment (T) *** ns * ns V × T ns ns ns ns

Values are shown in Mean ± SD.

CWL, continuous waterlogged; CD, continuous drought. $L_r$ (hydrostatic), hydraulic conductance driven by hydrostatic potential gradient; $L_p$ (hydrostatic), hydraulic conductivity driven by hydrostatic potential gradient; $L_r$ (osmotic), hydraulic conductance driven by osmotic potential gradient; $L_p$ (osmotic), hydraulic conductivity driven by osmotic potential gradient. The values followed by the same letter are not significantly different at $P < 0.05$ by Tukey’s test.

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**Figure 4.** Surface area of the whole root system of IRAT 109 and Taichung 65 grown under continuous waterlogged (CWL) and continuous drought (CD) conditions. Bars represent standard deviation ($n = 3$ plants). The values followed by the same letter are not significantly different at $P < 0.05$ by Tukey’s test.

**Figure 5.** The percentage of surface area of main root, L-type lateral root (L-type LR) and S-type lateral root (S-type LR) of IRAT 109 and Taichung 65 grown under continuous waterlogged (CWL) and continuous drought (CD) conditions. Bars represent standard deviation ($n = 3$ plants). The values followed by the same letter are not significantly different at $P < 0.05$ by Tukey’s test.
In this study, we firstly confirmed that heterorhizy exists based on differences in morphological and histological traits among the three component roots, which were evidenced by the observation of inner structure including outer layer of cortex, vascular bundle, root diameter, aerenchyma area (Table 2) and the observation of suberin lamellae and lignin depositions (Figures 2 and 3). We then attempted to identify the differential functional roles of each component root in the hydraulic architecture of rice root system. In relation to hydraulics, S-type LR had less cell layers, no aerenchyma, and less suberin lamellae and lignin deposition as compared with main root and L-type LR. These facts may contribute to reduce hydraulic resistance in radial water pathway, therefore, S-type LR was assumed to have the highest $L_p$ among the component roots.

As mentioned earlier, $L_p$ of the whole root system can be expressed as a product of the sum of $L_p$ of each component root and the composition of different component roots surface area. We then attempted to evaluate which term of the two has more impacts on the $L_p$ of the whole root system. In genotypic variations, $L_r$ and $L_p$ of IRAT 109 tended to show higher values than those of Taichung 65 (Table 3) while there were no traits that support such a difference, like less diameter, less aerenchyma formation, less suberin lamellae and lignin deposit in IRAT 109 than Taichung 65 grown under the respective same soil water condition. In addition, the CD treatment did not substantially affect the morphological and histological traits examined in both varieties. These facts imply either the differences in $L_p$ of each component root may not be the main factor that caused those in the $L_p$ of the whole root system, or those morphological and histological traits that were examined in this study may not have strong impact on $L_p$ of each root. We therefore need to examine further other traits that regulate the $L_p$ of each root like aquaporin activity.

In contrast, the second term, namely, the composition of different component roots surface area regulated more the $L_p$ of the whole root system. Specifically, IRAT 109 tended to show different composition of three component roots from Taichung 65 (Figure 5), and the CD treatment decreased total surface area of root system (Figure 4) and changed the composition of three component roots (Figure 5). These results show that these three component roots differ also in the developmental responses to soil water conditions which is also a quite important characteristic for heterorhizy (Suralta et al., 2018). Then, there were significant positive correlations between the percentage of surface area of S-type LR to the whole root system, and both $L_p$ (hydrostatic) and $L_p$ (osmotic) of IRAT 109 and Taichung 65 grown under CWL and CD conditions (Figures 6 and 7) whereas no such correlations were observed for the main root and L-type LR (Figures S1 and S2 in Supplementary Material). These results indicate that at the heading stage, S-type LR surface area primarily regulate $L_p$ of the whole root system and thus its water uptake ability.

Furthermore, it was found that the values of $L_p$ (hydrostatic) were higher than those of $L_p$ (osmotic) in both varieties and treatments indicating hydrostatic
potential gradient was the main driving force for water uptake of rice plant. However, it is very interesting that the percentage of surface area of S-type LR was positively correlated not only with \( L_p \) (hydrostatic) but also with \( L_p \) (osmotic). This result indicates that the function of S-type LR on water uptake is also important for water flow through cell-to-cell pathway.

Overall, this study demonstrated the differential functional roles of the three component roots in the hydraulic architecture of the rice root system by measuring both \( L_p \) (hydrostatic) and \( L_p \) (osmotic). Focusing on the differences among the component roots as heterorhizy, the results of this study strongly suggest that S-type LR has higher contribution to \( L_p \) of the whole root system than main root and L-type LR.

As for future studies, it is now important to directly measure the \( L_p \) of each of the component roots and further to examine aquaporin function, as water channels regulate the majority of water transport across cell membranes (Maurel et al., 2008) and this component could differ in each component root. Quantitative examination on the contribution of directly-measured \( L_p \) of each component root to that of the whole root system are further necessary to better understand the water uptake mechanism of the rice root system.

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