論文題目：Functional Roles of Heterorhizy in Water Uptake of The Rice Root System
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要約：

A rice plant develops a root system that shows unique morphological structure. It consists of morphologically different types of roots, including main roots (one seminal root and nodal roots), L-type lateral roots (L-type LRs) and S-type lateral roots (S-type LRs). L-type LRs that arise from the main roots as well as from the L-type LRs are generally thick and long, while S-type LRs that also arise from the main roots as well as from the L-type LRs are mostly thin and short. While, L-type LRs have the ability to branch into higher order laterals, S-type LRs do not have that ability. Thus, the root system is composed of different types of roots with various ages and such phenomenon is termed as heterorhizy. Those component roots have been reported to be different morphologically and anatomically, and therefore it can be assumed that they may play different regulations of hydraulic conductivity, which determines the overall water uptake ability by a whole root system. Based on the water flow model, which previously proposed by my study, when the hydraulic resistance is present in each component root which is reciprocal to hydraulic conductivity (Lp_r) of each component root, and are arranged in parallel, the Lp_r of the whole root system is the sum of products of Lp_r of each component root and the percentage of surface area of each component root to the whole root system. Since component roots have different morphological and anatomical features, I hypothesized that the different component roots have different hydraulic conductivities and their relative contribution to the overall Lp_r of the whole root system is different, and together will determine the water uptake ability of the whole root system. However, it is still unknown how each type of component root is involved in water uptake and transport as well as how they are different in their hydraulic properties, although the importance of lateral roots in water uptake as a whole has been recently brought to attention. Thus, the objective of this study was to determine the functional roles of each component root, and to evaluate their relative contribution to overall water uptake by the root system.

In the first study (Chapter 2), I have examined morphological and histological traits that are related to water uptake and transport of each component root and directly measured the hydraulic conductivity (Lp_r) of whole root system with a pressure chamber to quantitatively evaluate the relative contribution of different types of component roots to the overall water uptake of two rice varieties (IRAT 109 and Taichung 65). In this experiment, plants were exposed to continuous drought stress or were waterlogged, to measure Lp_r under these two contrasting soil water contents, and it was eventually confirmed that the drought treatment changed the relative composition of three types of component roots that included main roots, L-type LRs and S-type LRs as compared with the waterlogged control. Anatomical differences among component roots were also confirmed by making cross sections and I found that S-type LRs had less number and smaller diameter of xylem vessels as well as less number of cell layers in cortex than main roots and L-type LRs. Moreover, S-type LRs showed the absence of exodermis and aerenchyma formation whereas those structural features were confirmed in L-type LRs and main roots. Then, I found positive correlations between the percentage of surface area of S-type LRs to the whole root system of two rice varieties grown

until reproductive stage, and hydraulic conductivities (Lp_r) measured with pressure chamber. On the other hand, negative correlations were found for L-type LRs and the main root, which implies that S-type LRs had the highest contribution to overall water uptake of the whole root system among three different types of component roots.

To further confirm the positive contribution of S-type LRs to the Lp_r of the whole root system, it is effective to use the root materials that have a wide range of the target traits. In Chapter 3, therefore, I first examined the varieties and growing treatments that produce the wide variations in the target root traits (in section 1), and then determined such relationship at the single/individual root level (in section 2). In section 1, the developmental responses of component roots to osmotic stresses induced by 10% of PEG 6000 (-0.25MPa) were tested for six rice varieties and their contributions to water uptake were examined by the correlation between the percentage of surface area of different types of root to the whole root system and transpiration rate which is closely related to water uptake of the whole root system driven by hydrostatic potential gradient. As a result, surface area of the whole root systems were positively correlated with transpiration rates of plants grown under control and osmotic stress condition. Furthermore, transpiration rate of all rice varieties was significantly and positively correlated with the percentage of surface area of S-type LRs to the whole root system but not in L-type LRs nor main roots. These results suggest that S-type LRs had higher contribution to overall water uptake than L-type LRs and main roots. Among the six rice varieties, three varieties including IRAT 109, Swarna and Nipponbare which showed different root response to osmotic stresses, were selected for further measurement in section 2.

In section 2, I have measured the Lp_r of single main roots that contained both S-type and L-type LRs using a root pressure probe for above three varieties grown in aerated hydroponics and osmotic stress treatment (10% PEG 6000). Similar to Chapter 2, the relationships between the percentage of surface area of different types of root to the whole root with lateral roots and Lp_r were evaluated to confirm higher contribution of S-type LRs to overall water uptake at single/individual root level. Again, I found positive correlations between the percentage of surface area of S-type LRs to the whole root with lateral roots, and Lp_r for single/individual roots, which was consistent in the case of the whole root system as found in Chapter 2, while negative correlations were found for L-type LR. Thus, this experiment confirmed the higher contribution of S-type LR to the overall Lp_r at the single root level in addition to the whole root system.

In Chapter 4, I have analysed suberin deposition in the endodermis and exodermis as well as aquaporin activity in each component root, both of which have been shown to be important factors determining Lp_r. Aliphatic suberin amount was measured by using gas chromatography and mass spectrometry for each type of roots of three rice varieties used for Chapter 3. Even though, the same aliphatic suberin compositions were detected for both S-type and L-type LRs, the total amount was significantly smaller in both LRs compared with main roots of all varieties. Irrespective of the variety, S-type LRs had the lowest amount of suberin but was not significantly different from L-type LRs. For aquaporin analysis, I measured the expression level of *OsPIP2;4* and *OsPIP2;5* gene which has been reported to play crucial roles in fine adjustments of radial water transport in roots. In contrast, aquaporin expression levels differed among the component roots, and such differences also showed varietal differences. In IRAT 109, the expression was highest in S-type LRs among the component roots in control, but it was drastically reduced by the osmotic stress treatment. In control, the expression levels among the component roots were not significantly different in Swarna and Nipponbare, while under treatment, the expression level in S-type LRs was increased, and highest among the three component roots.

In the last study (Chapter 5), I further determined aquaporin activity in different types of component roots. Many studies have proposed that root aquaporins make a large contribution to whole plant water fluxes. Among the 33 aquaporin genes which have been identified so far, I have focused on *OsPIP2;1*, *OsPIP2;5* and *OsTIP2;1* in my study. These aquaporins except for *OsPIP2;1* are known as highly root specific genes. *OsPIP2;1* was recently reported to be one of the key genes among three *OsPIP1s* and four *OsPIP2s* regulating water uptake and plant growth. As aquaporins have been reported to be rapidly influenced by the surrounding conditions, root sampling method can be critical to obtain accurate data for gene, RNA and protein expression analyses. In general, samples are required to be immediately frozen in liquid nitrogen to avoid RNA degradation and preserve full-length RNA. For this reason, in most of the studies, the quantitative measurement of gene expression levels in roots is usually conducted by using the representative roots or the whole root system in a frozen condition because it is easier and faster to handle and collect such roots as compared with the use of samples of fine roots such as L-type and S-type LRs. On the other hand, in my study in which the heterorhizy is the key concept, it is required to separate roots of different types from each other, which is time-consuming and destructively remove lateral roots from a main root, which may affect the gene expression. Therefore, I firstly attempted to find an optimum condition of root sampling by measuring the gene expression with different sampling time length, and with or without shoot removal. Then, the expressions at mRNA and protein levels of selected three genes were measured for each type of roots of IRAT 109 with RT-qPCR and Western blot, respectively. Further, immunochemical analysis was conducted to confirm cell- and tissue-specific localization of these aquaporins in different types of component roots. The result showed no difference in gene expression level when root separation was conducted within 10 minutes after plants were taken out of the growth chamber with intact shoot. It was also found that S-type LRs had significantly higher expression levels of *OsPIP2;1* and *OsPIP2;5* genes than the other component roots. *OsTIP2;1* showed significantly higher expression in both L-type and S-type LRs than in main roots. In general, main roots exhibited relatively low levels of gene expression among the component roots for all the measured genes and these results consistent with protein levels. Moreover, immunochemical analysis revealed different localization of three tested aquaporin proteins in roots. OsPIP2;1 was detected on the entire tissue including exodermis but except for epidermis in all root types. OsPIP2;5 and OsTIP2;1 were not observed on the exodermis but they were detected on cortical sclerenchyma in main roots while OsTIP2;1 was less detected in the stele. Namely, OsPIP2;1 likely regulate water permeability at entire root tissue whereas OsPIP2;5 works in the continuous cell layers from cortical sclerenchyma to stele, and OsTIP2;1 only functions in the endodermis and cortical sclerenchyma. However, further studies require to clarify the localization of OsPIP2;5 in S-type LRs which the stain was not clearly detected in this study but showed greater expression at mRNA level in comparison to

L-type LRs and main roots.

In conclusion, this study showed the functional significance of heterorhizy and specifically that the roles in water uptake were different among the component roots. Namely, S-type LRs which have less cell layers, no exodermis and aerenchyma, less amount of aliphatic suberin in endodermis and high aquaporin activity, had the highest water uptake ability among the three component roots, and thereby contributed more to the water uptake of the whole root system. On the other hand, main roots and L-type LRs had larger xylem vessels than S-type LRs, which may indicate that they have larger axial conductance and may contribute largely to the transport of water to shoot. In addition, by forming aerenchyma which transports O_2 from shoot that is essential for branching and elongation of the roots, they contribute also to the water collection of the whole roots system by expanding its surface area.