論文審査の結果の要旨および担当者

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論　文　題　目

Physiological and Genetic Mechanisms of Lateral Root Formation for the Improvement of Root System Architecture in Rice

（根系構造改良に向けたイネ側根形成の生理・遺伝機構の解析）

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論 文 審 査 の 結 果 の 要 旨

Lateral roots (LRs) determine the overall root system architecture, thus enabling plants to efficiently explore their underground environment for water and nutrients. In rice plants, highly developed LRs confer improved performance and maintained productivity under stressful environments such as water-limiting and nutrient-poor soils. Recently, several genes controlling root development were identified, however, the genetic determinants and mechanisms regulating LRs remain largely unknown. In this dissertation, three studies were carried out aiming to determine the genetics and mechanisms of LR formation through the utilization of rice mutants and specifically focused on the characterization, expressions and functional analyses of their respective causative genes to understand the mechanisms regulating LR formation.

In the first study, we characterized a rice mutant, *wavy root elongation growth 1* (*weg1*), that produced a high number of long and thick LRs (L-type LRs) formed from the curvatures of its wavy parental roots caused by asymmetric cell growth in the elongation zone. Consistent with this phenotype, was the expression of the *WEG1* gene, which encodes a putative member of the hydroxyproline-rich glycoprotein family that regulates cell wall extensibility, in the root elongation zone. The asymmetric elongation growth in roots is well known to be regulated by auxin, but we found that the distribution of auxin at the apical region of the mutant and the wild-type roots was symmetric suggesting that the wavy root phenotype in rice is independent of auxin. However, the accumulation of auxin at the convex side of the curvatures, the site of L-type LR formation, suggested that auxin likely induced the formation of L-type LRs. This was supported by the need of a high amount of exogenous auxin to induce the formation of L-type LRs. These results suggest that the MNU-induced *weg1* mutated gene regulates the auxin-independent parental root elongation that controls the number of likely auxin-induced L-type LRs, thus reflecting its importance in improving rice root architecture.

In the second study, we newly isolated a *promoted lateral root* rice mutant, *plr1*, that also exhibited a higher density of L-type LRs but with normal and longer parental root growth than the wild-type. Remarkably, the mutant was characterized to have a significantly lower starch level in the stem while the glucose and sucrose levels at the root zone for early stage of LR formation up to the root tip were significantly higher as compared to the wild-type, pointing us to the involvement of carbohydrates in the regulation of LRs. This was supported by

the exogenous sugar (sucrose and glucose) treatments wherein *plr1* mutant seedlings increased L-type LR density, which may suggests that transported sugars are important for L-type LR formation. Interestingly, the wild-type seedlings also increased L-type LR density with glucose treatment. When wild-type seedlings were grown under nitrogen (N) conditions, the seedlings reduced stem starch levels resulting to increased L-type LR development via increased amount of glucose in the roots, providing further evidence that less stem starch accumulation and root sugar increase regulate L-type LRs. This low accumulation of starch in plr1 stem could be explained by the downregulation of the starch synthesis-related genes. At higher growth stage under N conditions, we further showed a strongly negative relationship of the stem starch accumulation to L-type LRs only among the root traits examined. Taken together, we reveal a mechanism to induce L-type LRs regulated through the involvement of less stem starch accumulation via increased root sugars both at seedling and higher plant growth stages of rice.

In the third study, we present the molecular mechanisms involved in the regulation of L-type LRs in *plr1* mutant apart from the above mechanisms of carbohydrates to induce L-type LRs. In here, the mutation gene of the *plr* mutant was revealed on Chromosome 9 and identified as *OsMED25*, which encodes a member of the Mediator complex that serves as a molecular bridge between gene-specific transcription factors (TF) bound at enhancers and RNA polymerase II, thus a key regulator of various developmental processes in plants. Consistent with the root phenotype, the *OsMED25* was expressed at the root cap and stele of the seminal and elongated LR roots as well as in the basal regions of LR primordia, suggesting the role of this gene in regulating the parental root and different stages of LR development. Additionally, we showed that OsMED25 possibly regulates these root components via auxin and that a high concentration of auxin is needed in the L-type LR formation. Furthermore, we showed that the mutation site is at the ACID protein domain of *OsMED25,* wherein this domain is important to regulate TF. By yeast-two hybrid system, the OsMED25 was shown to interact with the MYB TF. We speculated that this *MYB* TF gene negatively regulates L-type LR formation because the mutant did not interact with this TF and it produced L-type LRs. We further showed that the expression of *MYB* gene was downregulated under drought but it did not change under N conditions, which may suggest that *MYB* might be associated with the regulation of L-type LRs

under stressful environments but not under N conditions. Also, overexpression of *MYB* TF led to the greater expression of *MYB* gene and rendered the transgenic lines with significantly thinner diameter LRs, suggesting that *MYB* negatively regulates L-type LR formation. Collectively, our results suggest multiple roles of *OsMED25* and the participation of *MYB* TF in the regulation of L-type LR formation in rice.

Overall, this work provides newly identified genetic determinants and mechanisms in the regulation of LR development in rice through mutant analyses. The identified mutation genes that promote highly developed root system with no detrimental effects to the aerial parts are reported for the first time and thus the availability of *weg1* and *plr1*/*Osmed25* mutants represent important parent materials for genetic improvement and breeding purposes. We further demonstrated that nutrient levels determine enhanced LR development, hence, offering information on natural resource and crop management strategies for the improvement of root system architecture that could be useful for overcoming stressful conditions, such as drought, soil moisture fluctuations and nutrient deficiencies, especially in rainfed lowland and upland rice fields. Therefore, the examination committee of this doctoral thesis judged that the thesis has novelty, originality, and advanced academic values, and thus makes a substantial contribution to academic study in the relevant research fields.