主論文の要約

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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）

The root system is fundamentally important for plant growth and development because of its pivotal role in water and nutrient acquisition yet it has not received as much attention as the development of aerial plant organs until recently. It has been suggested that faster and more extensive root growth is important for plant survival in complex soil conditions and apparently strongly affect yield. In rice, the root system architecture (RSA) is determined by several root components such as seminal root, numerous crown roots and their LRs whose overall growth angle, elongation and branching through LR formation, reflects the structure and spatial configuration of the roots. Two types of LRs are distinctly recognize in rice: the L-type LRs, which are long, have a thick diameter and capable of higher order of branching, and S-type LRs, which are short, have a thin diameter and do not branch. It was suggested that RSA is a function of the plasticity in LR development particularly L-type LRs thus it is a major trait enabling plants to cope with various abiotic stresses and is therefore important in the genetic improvement for root system architecture in the current context of global climate change.

There has been gradual but significant progress made in the understanding of the genetic control of root development in rice through the use of mutants with specific defects on root morphological features and through the identification of QTLs using genetic linkage analyses, however, the genetic determinants and mechanisms regulating L-type LR formation are still unknown. In this manuscript, we present the genetics and mechanisms of L-type LR formation through the utilization of rice mutants. These mutants should be the first described so far in which the root morphological features are promoted specifically the frequency and branching of LRs and thus suitable for the molecular studies of LR development and should enable breeders to breed rice cultivars with enhanced RSA in the future.

We first isolated a rice mutant line, *weg1,* which exhibited a wavy parental root producing a

higher number of developed L-type LRs as compared to the wild-type plants, which displayed a straight parental root producing S-type LRs. In detailed observation, we found that these L-type LRs arose from the convex side of the curvatures of the wavy parental roots of *weg1* mutant. The causative gene of the *weg1* mutant is the *WEG1* gene, which encodes a putative member of the cell wall hydroxyproline-rich glycoprotein (HRGP) family, whose several members were reported to be regulating cell elongation. Thus, we thought that the *WEG1*, similar to the other HRGP members, also regulates cell elongation of the parental roots. This was confirmed in the expression analysis using qRT-PCR and GUS reporter, wherein the *WEG1* expression pattern was observed at the elongation zone and was highest at the cortical cell layers. This expression pattern is consistent to the asymmetric cell growth at the elongation zone whereby the cortical cell lengths at the convex (outer) side of the bent region were longer than the concave (inner) side. Therefore, *WEG1* controls normal HRGP regulation whereas HRGP regulation in *weg1* was abnormal, resulting to the parental root curvatures (bending). Despite, the wavy parental root phenotype of *weg1*, the seminal root length was similar to that of the wild-type because the cortical cell lengths at the convex side of the curvatures of *weg1* were similar to the cortical cell lengths at both sides of wild-type root and that the curvatures were not sharp curves. Moreover, it is well known that auxin regulates asymmetric elongation growth in roots, 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 mutant is independent of auxin. This auxin-independent parental root bending is causal for the production of L-type LRs in rice likely induced via auxin accumulation at the convex side of the bent region. Hence, the MNU-induced *weg1* mutated gene regulates the auxin-independent parental root elongation (bending) and indirectly regulated the L-type LR formation, that is likely dependent of auxin, thus proposing new mechanisms for the regulation of these traits. To attain enhanced LR development, manipulation of the parental root pattern from straight to wavy root would be important for increasing the number of L-type LRs.

On the other hand, we newly identified another mutant line, *plr1*, that displayed a higher number of L-type LRs with normal and longer parental roots as compared to the wild-type. Remarkably, *plr1* plant had significantly lower stem starch level, which coincided with the significantly higher root sugar levels especially at the root apical zone up to the early stage of LR development as compared to the wild-type plant, likely suggesting that less stem starch accumulation regulates L-type LR development via transport of sugars to the roots. This was supported by the exogenously applied sucrose and glucose to the roots that significantly increased the L-type LR density in the mutant, which may suggest that the transported sugars to the roots have promotive effects on LR development. Likewise, it was observed that the wild-type seedlings increased L-type LR density

with glucose application, which further supports that sugars have regulatory functions to promote L-type LRs in rice.

Because it was reported in *Arabidopsis thaliana* that N negatively affected starch accumulation in the shoot of the plant, we supposed that, in rice, less stem starch accumulation induces L-type LR development via sugar transport under N conditions. By using the wild-type, Nipponbare, we showed that the L-type LR density and total root length increased under N-applied conditions as compared to the N-deficient conditions. Concomitant to this, the stem starch accumulation was reduced and may have elicited the increased glucose levels in the zones for emerged and non-emerged LRs, suggesting the utilization of sugars for L-type LR formation and branching.

The *plr1* mutation gene might be involved in a novel sugar physiological action of both shoot and root on promoted LR development through the control of stem starch to increase root sugars for the promotion of L-type LR formation. We provided supporting evidences on the importance of the mechanism through the wild-type grown under N conditions. We further confirmed a strong negative relationship of stem starch accumulation to L-type LR traits only among the root component traits examined at higher plant stage. Taken together, our results highlighted the involvement of stem starch accumulation and root sugar levels to unravel the associations between shoot and root in the development of L-type LRs. Thus, these mechanisms propose the manipulation of the stem starch accumulation to induce L-type LRs for the improvement of root system architecture in rice.

However, we further observed that the wild type could not promote L-type LR density and length to the same level of the *plr1* mutant under N conditions, leading us to hypothesize that there might be another mechanism regulating L-type LR formation in the *plr1* mutant apart from the above mechanisms involving stem starch and root sugars. Thus, we aimed to identify the molecular mechanisms regulating the L-type LR development of *plr1* mutant. By map-based cloning procedure, a mutation site of the gene was found on Chromosome 9 that induced one amino substitution from glycine to glutamine at the ACID protein domain. The causative gene of *plr1* mutant is *OsMED25,* which encodes a subunit of the Mediator complex that interacts with transcription factors (TFs) through the ACID protein domain and thus this domain is important to interact with TFs. This may suggest that mutant could not interact with TFs while the Mediator complex in the wild-type interact with TFs and recruits RNA polymerase II for the transcription of target genes. We considered that the target TF negatively regulates L-type LRs because the mutant case produced high L-type LR number and the mutation of this gene did not interact with TFs. We show, in here, that *OsMED25* interacted with the TF, *MYB*, which we thought has negative regulation on LR formation. Moreover, according to the database (http://tenor.dna.affrc.go.jp), expression of MYB is dramatically down regulated under

drought, osmotic level and other stressful conditions, wherein rice plant can produce many L-type LRs. Similarly, rice plant produce L-type LRs under Nitrogen condition, however, the expression of MYB after N treatment did not change unlike the other conditions. This may suggest that MYB TF is very important to induce LRs under stressful conditions but not important under N. Thus, we focused on the molecular mechanisms and identified MYB as one of the interacting proteins regulating LRs. We further provided evidence that MYB is important in the regulation of LRs through the MYB over-expressed lines, whose L-type LR diameter was reduced.

As we described earlier, the L-type LR formation is a valuable trait for rice crop, because it accelerates access to water and nutrients at greater soil volume. Several studies have shown the importance of the promoted LR development for adaptation and productivity under changing soil environments. However, no genes regulating L-type LR formation were identified so far because there were no mutants with promoted LRs reported. Majority of the mutants have defects on root morphological features. This time, we utilized mutants with promoted LR development with normal and comparable or promoted parental roots, whose respective causative genes have no detrimental effects on shoot growth and yield despite the changes in parental root growth pattern and promoted LR development.

Furthermore, we evaluated the performance of the mutation genes under different conditions and we revealed that the L-type LRs are regulated by nutrient levels. When *weg1* mutant seedlings were grown under tapwater condition, they could not produce L-type LRs whereas they could produce L-type LRs under nutrient conditions. On the other hand, the root system of *plr1* mutant was evaluated under different N conditions (low, moderate and high) using rootbox method. The rootbox method was used to precisely observe the root system profiles of the 38-day-old *plr1* and wild-type plants. At harvest, the root system was harvested intact through pinboard sampling. We observed that *plr1* mutant had greater root system under low N conditions as compared to the wild-type and its performance was further enhanced under moderate and high N conditions. These results may imply that the mutation genes could be useful by understanding proper nutrient levels to regulate L-type LRs.

In the field, especially rainfed rice ecosystems (both lowland and upland), that depend on rainfall as source of irrigation, water scarcity is the major yield-limiting factor. Water stress significantly reduced plant growth and development. It also affects nutrient availability and mobility in the soil resulting to low nutrient use efficiency. Extensive root system has been considered as the major key trait in increasing productivity under these rainfed ecosystems because of greater access to limited water and even the immobile nutrients such as Phosphorous. Therefore, the utilization of these new rice mutant lines with promoted L-type LRs offer advantages in breeding program to help in the

development of stress-resilient rice varieties specific for these ecosystems. Moreover, timely N application (i.e. after sufficient rainfall), should be considered to further improve L-type LRs plasticity for a more vigorous root system, which will then be useful for the succeeding stressful condition such as drought and soil moisture fluctuations.