論文の内容の要旨

Functional analysis of the *DWARF WITH SLENDER LEAF1* gene that plays diverse roles in development of *Oryza sativa*

（イネの発生に多面的に作用する *DWARF WITH SLENDER LEAF1* 遺伝子の機能解析）

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Plant development is governed by the activity of the meristem. Lateral organs such as the leaf and flower are developed by cells supplied from stem cells at the peripheral region of the meristem. A lot of genes that regulate the meristem activity and the organ differentiation in plant development have been reported so far. However, the molecular mechanisms underlying the generation of the morphological diversities of angiosperms are not fully understood. Rice (*Oryza sativa*), a model plant of the monocots, had unique morphological characteristics. Rice leaves consist of the leaf blade and leaf sheath, which are connected by a boundary region (lamina joint), and have parallel longitudinal veins. In the reproductive phase, the inflorescence meristems generate branches, and then the branch meristems produce spikelets. A variety of genes

that regulate flower and inflorescence development have been identified, and the primary molecular mechanism such as that shown by the ABC model is conserved in rice. However, further researches are necessary to understand the developmental mechanism underlying the construction of morphology specific to rice plant. Thus, I aimed to elucidate a new gene that is involved in rice development and to gain some insights for understanding the function of genes related to distinct morphologies of rice. In this thesis, I focus on a novel rice mutant, *dwarf with slender leaf1* (*dsl1*), which exhibited pleiotropic defects in both vegetative and reproductive development.

In the vegetative phase, the lengths of both the leaf blade and leaf sheath were shorter in the *dsl1* mutant as compared to wild type. I also found that the width of the leaf blade was narrower in *dsl1* than in wild type. In association with the narrow width, both the number of the longitudinal veins and the distance between them were reduced in *dsl1*. My histological analysis suggested that a reduction in cell number rather than cell size seems to be a cause of the shorter distance between the longitudinal veins in *dsl1*. In addition, the reduced size of the vascular bundle also resulted from a decrease in the number of cells.

I hypothesized that the morphological defects of the leaf of the *dsl1* mutant are related to the abnormality of cell division. Then, I analyzed the spatial expression

pattern of *cyclin-dependent kinase B2* (*CDKB2*), a marker of the cell cycle. As a result, the level of *CDKB2* expression was reduced in the leaf primordia in *dsl1*. In addition, the number of cells expressing *CDKB2* was also decreased. These results suggested that the regulation of the cell cycle was partially disturbed in the leaf primordia of *dsl1*. I also found that the shoot apical meristem (SAM) shape in *dsl1* was slightly different from that in wild type. In consequence, the early stage of leaf development and the SAM maintenance seems to be affected in *dsl1*.

Next, I observed the phenotypes of the *dsl1* mutant in the reproductive phase. I first revealed that apparent semi-dwarfism in *dsl1* resulted from the shortening of the upper internodes, as compared with wild type. In addition, the internode patterning was unique in *dsl1*, because the elongation of the lower internodes was slightly promoted. I also found that the panicles and spikelets of *dsl1* were smaller than those of wild type. Moreover, some of the terminal spikelets replaced by abnormal appendages. These results suggest that the activities of both the inflorescence and branch meristems are reduced in *dsl1*. Furthermore, the *dsl1* spikelet showed low fertility and impaired anthers.

Gene isolation by the combination of positional cloning and next-generation sequencing revealed that *DSL1* encodes a histone deacetylase (HDAC) (Os04g0409600)

belonging to the Class I of the Reduced potassium dependence3 /Histone Deacetylase1 (RPD3/HDA1) family. To confirm that *DSL1* is Os04g0409600, I made and observed the knockout mutant of Os04g0409600 by using CRISPR-Cas9 technology. This knockout mutant represented the various morphological abnormalities similar to those observed in *dsl1*. Therefore, I concluded that *DSL1* is Os04g0409600.

To know the function of *DSL1*, I examined the organ-specific expression of *DSL1* by RT-PCR. Consistent with the pleiotropic alterations observed in *dsl1*, the expression of *DSL1* detected in a variety of organs and developmental stages.

In this thesis, I revealed that *DSL1* encodes an HDAC and plays various roles in rice development. To my knowledge, how HDACs contribute to the development of rice is poorly understood, and the *dsl1* mutant is the first mutant that reported as the loss-of-function of an HDAC of the RPD3/HDA1 family. In the future, it will be interesting to elucidate how DSL1 regulates such various developmental processes in rice and to identify the other proteins acting together with DSL1 and those controlled by DSL1.