論文の内容の要旨

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論文題目　**Studies on morphological and gene expression changes during leaf development in rice**
（イネの葉の発生過程における形態的及び遺伝子発現変化に関する研究）

Leaves are the most fundamental and crucial organs as the major sites of photosynthesis in higher plants. Leaf primordia are originated from the flanks of shoot apical meristem and undergo many complex developmental events; axis determination, cell proliferation, cell expansion, cell differentiation, and tissue differentiation. Various aspects of leaf development have been studied, and the mechanisms of spatiotemporal control of cell proliferation and cell differentiation are key events. Many genetic analyses for understanding these processes have been made, and most of the knowledge was obtained in a model eudicot, *Arabidopsis*. However, as the patterns of determination of developmental events differ among plant species, it is needed to study these processes in species other than *Arabidopsis*. Grass leaf can be an excellent model system for analyzing leaf development, because developmental and physiological events proceed from leaf tip to the base along the longitudinal axis of the leaf. However, there are few studies to clarify where the developmental events occur and how these events are regulated during leaf development in rice.

In this study, I examined rice leaves during morphogenesis to obtain basic information regarding the developmental transition by morphological, anatomical, and histochemical analyses. In addition, to understand

a whole picture of developmental transition of rice leaf, morphological changes caused by various factors and their links to gene expression pattern were analyzed.

1. Morphological and histological changes during rice leaf development

To determine the morphological changes that occur throughout rice leaf development, I separated mature and immature leaves from wild-type seedlings at 15 days after sowing (DAS) to examine the surface and internal structures of leaves. The leaf stage was defined as plastochron number; P1 represents the youngest primordium, P2 the next youngest, etc. Wild-type seedlings at 15 DAS contained eight leaves, P1 – P8. To understand the variation of surface structure of leaves, P1 – P6 leaf primordia were examined by scanning electron microscopy (SEM). SEM images revealed that the surfaces of P1 and P2 leaf primordia, and most part of P3 were covered with undifferentiated epidermal cells without any fine structure, but trichome initiation was observed on the epidermal cells at the apical part of the P3. P4 leaf primordium showed different epidermal structures along the apical–basal axis. At the apical-most part, fully differentiated cells were found on the surface. In contrast, this epidermal character was absent on the basal part of P4. On the most parts of P5 and P6 epidermis, mature differentiated cells were observed. Next, I examined the internal structure of P1 – P6. The results indicated that P1 and P2 primordia, and most part of P3 consisted of small cytoplasm-rich cells, and no tissue differentiation was observed. Cross-sections from the top to the base of the P4 revealed differentiated internal tissues at the top and middle part, and epidermal cells and mesophyll cells rich in cytoplasm at the basal part. Transverse sections of P5 and P6 leaves showed fully differentiated tissues along the leaf axis. These observations indicated that P1 to P3 leaf primordia are in the immature phase and P5 and P6 are in the mature phase of leaf development. A marked developmental transition would occur around the middle to basal part of the P4 leaf, and the cellular differentiation proceeded in a basipetal direction.

To obtain further evidence of the transition, I examined the cellular components of the P4 leaf; chloroplast and starch accumulation, and lignin deposition of the cell wall by histochemical analysis. Chloroplast autofluorescence was obvious in the apical and sub-apical parts of the P4. However, it was undetectable in the basal part of the P4. Numerous starch granules were detected in the basal part of the P4 leaf blade, but fewer starch granules were observed in the apical and sub-apical parts. Lignin accumulation was observed in the apical and sub-apical parts of P4, but it was not obvious in the basal parts of the P4 leaves.

These results indicated that the dynamic transitions of various aspects of developmental and physiological traits occur around the middle to basal part of the P4 leaf blade. The traits included the epidermal structure, internal structure and cellular components.

2. Gene expression changes during rice leaf development

To obtain a further understanding of the developmental transition and gradient along the leaf axis of P4 leaf primordium at the molecular level, I analyzed expression patterns of six marker genes—*OsCDKB2*,

OsRBCS2, *OsTCP1*, *OsTCP12*, *OsGRF10* and *pri-miR396c*—in eight segments from the top to the bottom of P4. In addition, I also examined the expression of these genes in three different stages; i.e., the early, middle, and late stages of P4. Higher levels of *OsCDKB2* and *OsRBCS2* expression would represent activation of cell division and photosynthesis, respectively. *TCP* and *GRF* genes are known to be involved in leaf development via regulating cell proliferation, and *pri-miR396c* is reported as a negative regulator of *GRFs*. Overall, the expression patterns of *OsCDKB2*, *OsGRF10*, and *OsTCP1* showed similar trends, which showed higher expression levels in the basalmost part of the P4. The *OsTCP12* displayed two peaks of expression at the basal and middle parts of the P4. *Pri-miR396c* was expressed in the basal part of the P4 in the late stages, but the highest level of expression was not observed at the basalmost part in the early and middle stages. The expression of *OsRBCS2* was highest at the apical part of P4 at the early stage of development, while the peak was not obvious in the middle and late stages.

The experiments showed that the expression peaks of marker genes shifted toward the basal part of the P4 leaf as developmental stage progress, indicating that these genes are influenced by developmental and/or physiological events that proceed in the basipetal direction. Thus, these six marker genes would be suitable to monitor developmental transition along the apical–basal axis of P4 leaves.

3. Effects of altered genetic factors on rice leaf development

I analyzed alteration of gene expression patterns in various mutants; *lg, dl, d61, d1, d18h, pla1-4* and *pre.* These mutants show abnormalities in leaf morphology. The expression patterns of five marker genes were unchanged in *lg*, and that of *OsCDKB2*, *OsGRF10* and *OsTCP1* were not obviously altered in all mutants. In contrast, the expression pattern and the level of *OsTCP12* and *pri-miR396c* were affected in most of the mutants. Expression pattern of *OsTCP12* was affected in *d61* and *d1* mutants and the expression level was altered in *dl*, *pla1-4* and *pre*. Expression pattern of *pri-miR396c* was affected in *d1* and the expression level was altered in the others. In case of *d18h*, expression trends of *OsCDKB2*, *OsTCP1* and *OsGRF10* would be similar to that of wild-type despite of the reduced length of the P4. Consequently, the results indicated that expression patterns and level of the marker genes were affected in *dl, d61, d1, pla1* and *pre* mutants, but not in the *lg* mutant. The trend of the expression profile in *d18* was globally conserved compared to that of the wild-type controls. The results indicated that alteration of marker gene expressions differed among the mutants, and the sensitivity of *pri-miR396c* and *OsTCP12* expression changes was high, while that of *OsCDKB2*, *OsGRF10*, and *OsTCP1* was low.

Accordingly, my expression analysis using six marker genes would be useful to characterize developmental features of mutants and to understand the genetic and physiological alterations that could not be determined from the morphological phenotype.

In conclusion, I characterized the developmental progression of wild-type leaves. The results revealed the dynamic transition of developmental events, in particular, from cell proliferation to cell differentiation in the P4 leaf primordium. I developed several molecular markers showing dynamic expression from tip to base of P4, and applied them to various morphogenetic mutants. The changes in expression patterns of marker genes varied among the mutants, indicating that my marker genes are useful for detecting effects on the transition process from cell proliferation to cell differentiation during leaf development. The results would provide basic knowledge regarding the developmental transition and effects of various factors on the transition process.