論文内容の要旨

論文題目　Studies on the molecular mechanism of spatial vein pattern formation in rice.

（イネにおける空間的な葉脈パターン形成の分子機構の研究）

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In higher plants, the vascular system plays a crucial role in transport of not only water and nutrients but also signal molecules. For this role, the vascular system forms a well-organized network through the plant body. The vascular pattern organization has been considered as a paradigm of tissue pattern formation in higher plants and then has been studied by a number of researchers. However, in comparison with studies of eudicot such as *Arabidopsis thaliana*, less is known about regulation of vascular pattern formation in monocots. In this thesis, therefore, I studied a regulation of leaf vascular pattern formation in monocots using rice as a model.

First, I observed in detail the process of vascular tissue development by making serial sections of the basal region of leaves from two monocotyledonous species, *Oryza*

sativa (rice) and *Zea mays* (and maize). This observation revealed that the differentiation process in large vascular bundle (LV) was divided into six stages in rice and five stages in maize; I) emergence of procambium strands in the middle of three ground meristem cell layer and establishment of circular layer in outermost zone of procambium strand; II) emergence of protoxylem and phloem tissue according to the dorsoventral axis in leaves; III) differentiation of secondary protoxylem; and IV) emergence of two metaxylems adjacent to the circular layer between protoxylem vessels and phloem tissue. Although rice and maize showed similar differentiation process until stage IV, in stage V, the differentiation process in LV differed in rice and maize. In rice, ground meristem cells surrounding the circular layer started expanding and differentiating into vascular bundle sheath cells in stage V, and two sheath structures were established in stage VI. On the other hand, the circular layer in maize kept on expanding and turned into vascular bundle sheath in stage V. This difference in the differentiating process caused the structural differences in LV between rice and maize.

The differentiating process of small vascular bundle (SV) in rice and maize showed basically the same process as that of LV. From observation of differentiating commissural veins (CVs) in rice and maize, the initial position of CV was elucidated in longitudinal veins. In rice and maize, CV occurred in a circular layer cell that usually kept in touch with both a metaxylem cell and phloem tissue in stage V. This result suggested the relationship between the maturation of longitudinal veins and CV formation, in other words, the existence of cell–cell interaction in vascular pattern formation in monocotyledons.

Secondly, based on these observations, I screened mutants exhibiting altered vascular pattern in rice leaves and isolated eleven mutants. Of them, one mutant showed short CV intervals and in severe cases clustered CVs caused by excessive formation, and I named as *commissural vein excessive1-1* (*coe1-1*). This novel phenotype was thought to provide us the advance of our knowledge in vascular pattern formation. Detailed observations in the cell length along the proximodistal axis revealed that short CV intervals in *coe1-1* were independent from cell elongation in leaves. Because *coe1-1* mutant indicated several phenotypes besides short CV intervals, causal gene of *coe1-1* mutant was predicted to be a factor that would contribute to several signaling pathways. Positional cloning and genetic complementation revealed that *Os08g0442700* was the *COE1* gene and *Os08g0442700* encoded a leucine rich repeat receptor-like kinase (LRR-RLK). PFG_2A-30259 and RMD_04Z11AQ65 had a T-DNA insertion in *Os08g0442700* ORF, and both lines showed short CV intervals and clustered CVs like *coe1-1* mutants. Therefore, PFG_2A-30259 and RMD_04Z11AQ65 were referred as *coe1-2* and *coe1-3*, respectively. For proper evaluation of CV pattern formation, the intervals between two CV initiation points (CVIPs) were measured and were plotted on a histogram. In three wild-type backgrounds of *coe1* mutant alleles, the distribution curve of CVIP distances were almost the same. All *coe1* mutant alleles showed increase in short CVIP distances less than 50 μm although wild-type plants did not show such short CVIP distances. *coe1-1*, a weak mutant allele showed a shift of the distribution curve to a shorter position, but *coe1-2* and *coe1-3*, strong mutant alleles lacked obvious peak in the distribution curve. These results suggested that COE1 would function in both regulation of CV intervals and suppression of clustered CV formation. Next, analysis of the effects of auxin signaling on CV formation revealed that CV intervals in wild-type plants widened in proportion to exogenous auxin levels. The *coe1* mutants showed insensitivity to auxin signaling only in CV formation. This result suggested that COE1

would function in CV formation under auxin signaling pathway. On the other hand, PAT inhibitor applications to wild-type plants did not produce clustered CVs observed in the *coe1* mutants.

Finally, I investigated the relationship between the *coe1* mutants and brassinosteroid (BR) signaling. Under continuous darkness, the *coe1* mutants showed reduced elongation in internodes and mesocotyl and this defect is known to be a typical BR-deficient phenotype. However, other BR-dependent events such as leaf elongation, root elongation, bending angle of lamina joint was normal. These results suggest that COE1 may partially function under the BR signaling pathway. In CVIP histogram analysis, the *d61* mutant, which is defective in BR perception, showed extended CV intervals, suggesting, that perception of BR signaling may be related to CV pattern formation. Then, the wild-type and *coe1-2*, the strongest allele was treated with brassinolide (BL), an active BR, and brassinazole (BRZ), an inhibitor of BR biosynthesis. Exogenously applied BL and BRZ made CV intervals wider or narrower in wild-type, respectively. In contrast, *coe1-2* showed insensitivity to both BL and BRZ. These results indicated that the CV intervals are regulated by BR thorough COE1. Interestingly, *coe1-1*, a weak mutant allele exhibited the BRZ-induced enhancement of clustered CV formation. This BRZ-induced enhancement of CV clustering was suppressed by simultaneous application of BL. These results suggest that clustered CV formation is also related to BR signaling. Taken altogether, my results indicate that COE1 regulates adequate CV intervals directly or indirectly through BR signaling.

In conclusion, CV formation was controlled through a novel receptor like kinase, COE1 under both auxin and BR signaling.