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# 主 論 文 の 要 旨

論文題目　Three-dimensional analysis on the internal structure of leaf tissue in rice
（イネ葉組織内部構造の三次元解析）

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# 論 文 内 容 の 要 旨

Anatomical characteristics of the mesophyll in leaves are essential for understanding the photosynthetic ability, potential productivity, and environmental stress adaptation. Rice (*Oryza sativa* L.) has smaller mesophyll cells (MC) than other crop species, and the cells have a large volume of chloroplasts. The cross-sections of rice leaf tissues show MC of various sizes and shapes without distinction of palisade or spongy cells. Moreover, different MC structures are observed in transversal and longitudinal sections of rice leaf tissue. The surface area of MC ($S_{mes}$) and chloroplasts ($S_c$) facing intercellular airspace (IAS) are key parameters for estimating photosynthetic capacity from leaf anatomy. Although the $S_{mes}$ and $S_c$ are calculated based on the shape assumption of MC, it is questionable if the assumption is correct for rice MC with concave-convex surfaces. Furthermore, rice is sensitive to salt stress, which causes structural changes in chloroplast. Therefore, the three-dimensional (3D) reconstruction of MC in wide range of leaf tissues is necessary to elucidate the whole leaf anatomy of rice. However, understanding of MC structure in whole leaf tissue of rice is still limited due to the lack of methods that allow for extensive analysis, including subcellular information. In my doctoral dissertation, I aimed to elucidate MC structure in whole leaf tissue of rice and to uncover the structure-function nexus of MC. To achieve the aim, I established the 3D reconstruction method using light microscopy to detect the MC in whole leaf tissue (chapter 1). Then, I utilize the 3D reconstruction to determine the connection of MC structure to an anatomical parameter using the different sectioning orientations of the leaf tissues (chapter 2). Finally, I applied the 3D analysis to elucidate the MC structure in different positions in leaf tissue and determine how salinity affects the MC structure in each position (chapter 3).

In chapter 1, establishing the serial section light microscopy (ssLM) method followed by the 3D reconstruction provides virtual sectional images at various angles, overcoming the conventional light microscope method. The wide ranges of 70–90 μm thickness of rice tissue have been observed and reconstructed into the 3D models that allow evaluation of the structure of MC and internal chloroplasts. Furthermore, the coverage of the chloroplasts on the cytoplasm periphery was quantitatively evaluated. Although the light microscope resolution is lower than the electron microscope, ssLM allows observing the broad scale of leaf tissue with the information on the subcellular structure.

In chapter 2, I further developed the ssLM method to detect the IAS, MC, and chloroplasts in rice leaf tissue and reconstructed the structures into 3D models. The obtained 3D models revealed that volumes of IAS and MC accounted for 30% and 70% of rice leaf tissue excluding epidermis, respectively, and volume of chloroplasts accounted for 44% of MC. The actual $S_{mes\text{-}3D}$ and $S_{c\text{-}3D}$ calculated from the 3D models revealed that the shape-specific assumption on the sectioning orientation affected the estimation of $S_{mes}$ and $S_c$ using 2D section images with discrepancies of 10 – 38%. This result suggested that the sectioning orientation affects the estimation of $S_{mes}$ and $S_c$ in rice leaf tissues. I conclude that the most accurate way to estimate $S_{mes}$ and $S_c$ from the 2D section images of rice leaf tissue is to use the longitudinal sections and assume that MC is an oblate spheroid.

In chapter 3, from the 3D analysis, I found that rice leaf tissue has different structures of MCs with greater diversity in adaxial and abaxial cells. These cells showed different responses to salinity stress. The middle MC appeared to be an ellipsoid disc with several lobes on the cell periphery, which is consistent with the typical rice MC structure. The adaxial and abaxial MC located close to the leaf surface appeared to have a more varied structure. The adaxial MC appeared to be an ellipsoid stand with projection on the cell periphery and had higher chloroplasts than MC at the middle and abaxial positions. The salinity stress reduced the size and height of MC and coverage of the chloroplast on the cytoplasm periphery at the adaxial and abaxial positions, as well as the chloroplast size of adaxial MC. These results suggested that chloroplasts in the MC close to the epidermal cells tend to be more damaged.

Taken together, the complicated MC shape of rice was revealed using ssLM followed by the 3D reconstruction, suggesting the underestimation of the 2D sections associated with the shape assumption of MC on the sectioning orientation of leaf tissue. The 3D reconstruction models of MC throughout rice leaf tissue revealed differences in structure depending on position and different responses to the salinity stress.