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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Summary

Rice (*Oryza sativa* L.) is a monocotyledonous grass that does not form palisade and spongy cells, and the intricated shape of mesophyll cells (MC) with the large volume of chloroplasts allows to enhance CO_2 diffusion. In addition, rice is sensitive to salt stress that induces changes in MC structures. The 2D cross-sections of rice leaf tissues show MC of various sizes and shapes. 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 airspaces (IAS) influenced CO_2 diffusion from stomata to the carboxylation sites. The S_{mes} and S_c are calculated based on the shape assumption of mesophyll cells on 2D sections. However, the concave-convex surfaces of rice MC are questionable to assume the shape correctly. Although recently, three-dimensional (3D) analysis of leaf anatomical traits has become the implication for assessing photosynthetic capacity, the observation on wide range of leaf tissue with subcellular information is still limited due to the lack of methods that allow extensive analysis. 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 in Chapter 1, I established the 3D reconstruction method using light microscopy to detect the MC in whole leaf tissue. In Chapter 2, 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. Finally, in Chapter 3, 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.

In Chapter 1, I established the 3D reconstruction method based on serial section light microscopy (ssLM) to analyze a wide range of structures in rice leaf tissue and their intracellular

structure. The 3D reconstruction based on the ssLM method 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 structure 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. Actual S_{mes} and S_c values estimated from 3D model were compared with the conventional method for 2D images to find the correct shape assumption of rice mesophyll cells in the different orientations of leaf tissue. 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 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 tissue. I concluded 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 the mesophyll cell is an oblate spheroid.

In Chapter 3, I divided the mesophyll tissue of control and salt-treated leaves into adaxial, middle, and abaxial positions, and eight mesophyll cells were selected from each position and reconstructed into 3D models. The anatomical traits of each position in control and salt-treated leaves were calculated based on the 3D models. From the 3D analytical approach, I found that differences structure thaof 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 response to the salinity stress.