論文内容の要旨

# Analyses of the changes in stomatal and mesophyll $CO_2$ diffusion conductances in response to the atmospheric $CO_2$ concentration or soil water content

（気孔および葉肉における $CO_2$ 拡散コンダクタンスの $CO_2$ 濃度と土壌水分量への応答の解析）

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$CO_2$ diffuses from ambient air to the chloroplast stroma. There are two large resistances in this diffusion pathway, stomatal resistance ($r_s$) and mesophyll resistance ($r_m$). $r_s$ is the resistance from the leaf surface to the intercellular air space through stomata. $r_m$ is the resistance from the intercellular air space to the chloroplast stroma. $CO_2$ concentration is highest in the air ($C_a$), and lowered in the intercellular air space ($C_i$) and lowest in the chloroplast stroma ($C_c$), because of substantial $r_s$ and $r_m$. These resistances are often expressed as conductances, inverse of resistances, $g_s$ and $g_m$.

These conductances respond to various environmental changes. In this thesis, I conducted detailed analyses of $CO_2$ diffusion conductances in response to drought and $CO_2$ concentration.

At first, I sought for factors that decrease $g_m$ under drought conditions. ABA was one of candidates that would cause the decrease in $g_m$. Therefore, I used an ABA deficient mutant (*aba1*) and the wild type of *Nicotiana plumbaginifolia*. These plants were exposed to drought conditions to investigate whether the increase in ABA content in the leaves was needed for the decrease in $g_m$. For the $g_m$ measurements, I constructed a special system to measure $g_m$ with high accuracy using the carbon isotope method that is considered as most reliable. Under drought conditions, *aba1* did not show any decrease in $g_m$ whereas $g_m$ decreased in WT. Addition of ABA to *aba1* leaves caused dramatic decreases in $g_m$. I, thus, could demonstrate that the increase in ABA content in the leaf was necessary for the decrease in $g_m$. However, the underlying mechanisms are still not clear.

In addition to this experiment, I investigated whether $g_m$ responded to high $CO_2$ condition with these tobacco plants because some papers have reported rapid decrease in $g_m$ in response to high $CO_2$. In both WT and *aba1*, $g_m$ decreased in response to high $CO_2$. Therefore, ABA might not be necessary for decrease in $g_m$ in response to high $CO_2$.

There are only a few papers reporting detailed analyses of responses of $g_m$ to elevated $CO_2$. In particular, studies reporting responses of $g_m$ to long-tem elevation of $CO_2$ are few. Because, when the stomata close, Rubisco tends to fix more $CO_2$ evolved in the process of (photo)respiration than the $CO_2$ directly from the ambient air, I used some stomatal mutants of *Arabidopsis thaliana,* which are insensitive to $CO_2$, to uncouple the influence of $g_s$ on $g_m$. To estimate $g_m$, I also applied new methods that

were proposed very recently. The plants were grown at 390 ppm and 780 ppm in growth chambers to investigate whether the responses of $g_m$ to elevated $CO_2$ could be changed by growth $CO_2$ concentration. In the short-term experiments, $g_m$ decreased in response to elevated $CO_2$ regardless of $g_s$ responses and the calculation methods to estimate $g_m$. In the long-term experiment, the responses of $g_m$ to elevated $CO_2$ did not change with the growth $CO_2$ concentration. However, nitrogen nutrition during the growth affected responses of $g_m$ to elevated $CO_2$. The difference might be due to changes in chloroplast starch metabolism. With the decrease in $CO_2$ concentration and/or nutritional N level, starch tended to accumulate, which would decrease $g_m$.

I investigated underlying mechanisms of the changes in $g_m$ in response to elevated $CO_2$ and ABA. Recently, some studies have suggested that the PIP aquaporins could affect $g_m$. Therefore, to clarify whether PIP aquaporins are involved in the changes in $g_m$ in response to elevated $CO_2$ and ABA, I compared responses of $g_m$ to elevated $CO_2$ and ABA among three T-DNA insertion lines of PIP aquaporins that are highly expressed in leaves (*pip1;2*, *pip2;3* and *pip2;6*). The responses of $g_m$ to elevated $CO_2$ were all the same among Col-0 and all T-DNA insertion lines. However, in *pip2;6*, $g_m$ was insensitive to ABA. As PIP2;6 was mainly expressed around the vascular tissue, PIP2;6 would not play roles in mesophyll cells as $CO_2$ facilitators. Previous reports have demonstrated that the relationships between leaf water relations and PIP aquaporins. Then the changes in the water relations would affect $g_m$.

Clarification of the relationships between leaf water relations and $CO_2$ diffusion in the leaves will be prerequisite to improve plant performance in semiarid and arid areas.

Also, detailed analyses of responses of $CO_2$ diffusion conductances to high $CO_2$ will be helpful to improve plant performance in the high $CO_2$ world. The results are discussed in the light of these future perspectives.