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Research Background

Presentation Title: *in vivo* Rubisco Kinetics in Rice

Abstract:
Yield increases in agriculture are needed to feed the growing world population. Rice is a staple crop for much of the world's population. The two main photosynthetic pathways that plants utilize are termed C_3 and C_4. Plants that utilize the C_4 pathway have higher photosynthetic capacities, are more water and nitrogen use efficient, and generally have greater yields. Currently, rice yields are limited by photosynthetic capacity; therefore, there is a large research effort, called the C_4 Rice Project, directed toward improving yields by introducing the C_4 photosynthetic pathway into rice. If the C_4 pathway is successfully introduced into rice, then the properties of the photosynthetic enzyme Rubisco will likely need to be optimized for the C_4 system in order to maximize rice yields. However, the kinetic properties of Rubisco in rice are not known, making it difficult to predict how it will perform in a C_4 system. The aim of my project is to define the kinetic parameters of rice Rubisco with *in vivo* gas exchange measurements. Rice Rubisco kinetics and the conductance to CO_2 movement within a leaf were determined at 25 ^{o}C and compared to previous *in vivo* measurements.

Research Context/Background:

Yield increases in agriculture are needed to feed the growing world population. Rice (*Oryza sativa*) is a staple crop for much of the world's population, providing a fifth of the total calories consumed by people worldwide (Smith, 1998). The two main photosynthetic pathways that plants utilize to do photosynthesis are termed C_3 and C_4. The C_3 pathway takes up carbon directly from the air and incorporates it into plant matter. C_4 plants however, have a concentrating mechanism for carbon dioxide (CO_2) within the leaf that they utilize before fixing atmospheric carbon into sugars. Plants that utilize the C_4 pathway have higher photosynthetic capacities, a more efficient use of water and nitrogen, as well as greater yields (Caemmerer, 2012). Currently, rice yields are limited by their photosynthetic capacity (Furbank, 2009); therefore, there is a large research effort, called the C_4 Rice Project, directed toward improving yields by introducing the C_4 photosynthetic pathway into rice (Caemmerer, 2012). If the C_4 pathway is successfully introduced into rice, then the properties of the photosynthetic enzyme Rubisco will likely need to be optimized for the C_4 system to maximize yields. Because Rubisco is the first enzyme involved in fixing CO_2 into the plant, its speed and affinity for substrates (kinetic properties) largely determine the rates of photosynthesis. However, the kinetic properties of Rubisco in rice are not known making it difficult to predict how the enzyme will perform when placed into a C_4 photosynthetic system.

The long-term goal of the C_4 rice project is to optimize rice photosynthesis for maximum productivity (Covshoff, 2012). However, Rubisco is a slow and non-specific enzyme that catalyzes both the oxygenation and carboxylation of Ribulose-1,5-bisphosphate. The products of the carboxylation reaction are used in photosynthesis, while the products of the oxygenation reaction must be recycled by photorespiration, costing the plant carbon and energy. There has been an observed trade-off where a Rubisco that has a high turnover rate will also have a low specificity for CO_2 over oxygen (O_2), resulting in increased rates of photorespiration (Sage, 2002). Because Rubiscos that have evolved under a C_4 system experience a high CO_2 environment, it is predicted that they will have a higher turnover rate and lower specificity than C_3 Rubisco. This is because the high CO_2 environment within the leaf minimizes any reaction with O_2. This would suggest that a C_3 Rubisco placed in a C_4 system would not be efficient given that it would have a lower turnover rate, resulting in overall lower photosynthetic rates than other C_4 plants. The current lack of knowledge regarding Rubisco kinetics leave a gap in understanding the effects of having rice Rubisco present within a C_4 system. To address this knowledge gap, I measured the *in vivo* kinetics of Rubisco in rice at 25 ^{o}C.

Successful completion of this project required the use of plants with genetically reduced Rubisco content within the leaf. This allowed for photosynthesis to be determined by Rubisco kinetics across all measured CO_2 concentrations. The specific aims of this project were to: 1) characterize the plants as having decreased levels of Rubisco, 2) measure *in vivo* kinetics, and 3) to measure the conductance of CO_2 to the site of Rubisco carboxylation. Completion of my first aim allowed me to determine if the plants I was working with had sufficiently low levels of Rubisco in order to measure kinetics. My second aim of measuring Rubisco kinetics allowed me to compare C_3 rice to other C_3 plants with published kinetic data. My third aim of calculating CO_2 conductance within the leaf is important for quantifying how much CO_2 is present at the active sites of Rubisco. Because CO_2 must diffuse through the epidermis, cell wall, and plasma membrane in order to reach the chloroplast where Rubisco is located, estimations of the concentration of CO_2 surrounding Rubisco must account for the differences between the CO_2 within the leaf and the air surrounding it. This measurement of CO_2 conductance is important for insuring that my calculations of Rubisco kinetics are accurate.

The results of my project have been to identify two lines with sufficiently low Rubisco concentrations, to obtain Rubisco kinetic parameters for rice, and to calculate mesophyll CO_2 conductance at 25 ^{o}C. The data gathered thus far matches with *in vivo* data collected for another C_3 species (Table 1), which further indicates that this enzyme would not be optimal in a C_4 system. In the future, a complete temperature response of Rubisco kinetics needs to be measured in rice from 10 to 40 ^oC. Additionally, the derived Rubisco kinetic parameters for rice will be used to model the ability of this enzyme to support C_4 photosynthesis.

Table 1: Comparison of kinetic data and conductance values from Tobacco (von Cammaerer et al., 1994) and from this project on rice. Presented are five important parameters related to Rubisco kinetics: the CO_2 conductance (g_w), the Michaelis-Menten constants for CO_2 and O_2 (K_c and K_o, respectively), the ratio of maximal rates of oxygenation and carboxylation (V_{omax}/V_{cmax}), and the CO_2 compensation point Γ*.

Species	g_w (mol m^{-2} s^{-1} bar^{-1})	K_c (μbar)	K_o (mbar)	V_{omax}/V_{cmax}	Γ* (μbar)
Tobacco (*Nicotiana tabacum*)	0.3	259 ± 57	179	0.255	38.6
Rice (*Oryza sativa*)	0.08	302 ± 41	296 ± 74	0.377	37.9

Acknowledgments:
Special thanks to the WSU College of Arts and Sciences for financial support as well as funding from NASA's Space Grant Consortium and the Auvil Scholar Fellowship. Additionally, support was provided by the International Rice Research Institute, The Philippines, C_4 rice program, through a grant by the Bill and Melinda Gates Foundation, and also by the UK Department for International Development and Instrumentation obtained through an NSF Major Research Instrumentation grant to Dr. Cousins. This project could not have been completed without assistance from Charles Cody in setting up growth conditions and monitoring growth chambers for the duration of the experiment. Additional thanks to Berkley Walker and Ryan Boyd in teaching and assisting with gas exchange measurements. Seeds were generously provided by Professor Amane Makino in Graduate School of Agricultural Science at Tohoku University Japan.

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Faculty Endorsement

Name of Faculty Advisor: Dr. Asaph Cousins, Assistant Professor, School of Biological Sciences

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Faculty Endorsement: I am happy to write a letter acknowledging Thomas Sexton's fine work over the last year in my laboratory. Mr. Sexton has been working in the lab in collaboration with a graduate student and post-doc collecting an array of molecular, physiological and biochemical data. He is dedicated to his project and has collected a significant amount of data that I hope will be published in 2014. Already, Mr. Sexton has given three poster presentations on his research accomplishments and won best undergrad poster at the Molecular Plant Sciences retreat at WSU. The anticipated outcomes from his project will be the first ever *in vivo* Rubisco kinetics data for a grass species, which on its own will be invaluable for modeling photosynthesis in crop species under future climatic conditions. Additionally, Mr. Sexton's data set will be essential for accurately predicting the feasibility and success of introducing C_4 photosynthesis in *O. sativa* as part of the C_4-Rice research currently supported by the Bill and Melinda Gates Foundation and the International Rice Research Institute.