Heightened Frontiers in Plant Biology

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Final Program

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Malate is crucial for symbiotic dinitrogen (N_2) fixation, occurring in high concentrations in N_2 fixing nodules as the major carbon source for bacteroid respiration. Malate also provides carbon skeletons for the assimilation of fixed nitrogen from ammonia into amino acids and is proposed to be involved in the regulation of the nodule oxygen diffusion barrier, important for the maintenance of nitrogenase. An alfalfa nodule enhanced malate dehydrogenase (neMDH; EC 1.1.1.37) with high affinity for the reduction of oxaloacetate to malate, was over expressed in transgenic alfalfa using the CaMV35S promoter. Over expression of the transgene increased neMDH transcript abundance in leaves and roots. Western blots indicated that neMDH protein concentration was increased in root and leaf tissue of transgenic alfalfa. Experiments using $^{15}N_2$ labeling confirmed that transformed alfalfa had significantly higher N_2 fixation rates (0.406 nmol N_2 hr^{-1} $plant^{-1}$) than control plants (0.257 nmol N_2 hr^{-1} $plant^{-1}$). Enhancing the expression of genes involved in malic acid synthesis appears to be a suitable strategy to increase nitrogen fixation in legumes.

591 Session 60, Plant Pathogen/Symbiont Interactions

Identification and characterization of *Medicago truncatula* mutants impaired in the development of an arbuscular mycorrhizal symbiosis. Blaylock, Laura Harrison, Maria *The Samuel Roberts Noble Foundation Presenter: Blaylock, Laura lablaylock@noble.org*

Arbuscular mycorrhizal (AM) associations are symbiotic associations formed between fungi and higher plants. The fungi colonize the root in order to obtain carbon from their host; while the fungi, in turn, assist the plant with mineral nutrient acquisition such as phosphate. We are using a model legume, *Medicago truncatula*, and an AM fungus, *Glomus versiforme*, to study the molecular mechanisms underlying the symbiosis. The aim of this project is to identify mutants of *M. truncatula* that are unable to form complete arbuscular mycorrhizal associations. From a screen of a *M. truncatula* EMS population, we isolated two mutants which allowed *G. versiforme* to enter the root and form internal structures. However, the phenotype differed from the wildtype in that the volume of root that was colonized was significantly lower than wildtype and the ratio of internal hyphae to arbuscules was increased. In addition, in one mutant, the arbuscules were stunted and many of the hyphae were septate, suggesting that the fungus was dead or dying. Vital staining of the internal fungal structures supported this hypothesis. The mutants were backcrossed to the wildtype and subsequent progeny screening is in progress. The mutants were crossed to each other, and the F_1 phenotype was wildtype indicating that they are not allelic. Northern blots of root RNA showed noticeable differences in the expression of a selection of genes. We are currently working toward mapping the genes responsible.

592 Session 61, Photosynthesis

Physiological functions of the water-water cycle (Mehler reaction) and cyclic electron flow around PS I in rice leaves. Makino, Amane *Graduate School of Agricultural Sciences, Tohoku University* Miyake, Chikahiro *Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University* Akiho, Yokota *Graduate School of Biological Sciences, Nara Institute of Science and Technology Presenter: Makino, Amane makino@biochem.tohoku.ac.jp*

Changes in chlorophyll fluorescence, P-700$^+$-absorbance and gas exchange during photosynthetic induction from darkness to high irradiance were examined simultaneously on rice (*Oryza sativa* L.) including the *rbc*S antisense plants. Whereas ϕPS II increased more rapidly than CO_2 assimilation at 20% O_2, there was no difference between them at 2% O_2. Because of its dependency on O_2 concentration, extra electron flow at 20% O_2 was regarded as the water-water cycle (WWC). The WWC reached maximum just after illumination and led to a rapid formation of non-photochemical quenching (NPQ). Then, WWC and NPQ decreased gradually with increasing CO_2 assimilation. During photosynthetic induction at 2% O_2, ϕPS I was always higher than ϕPS II, suggesting that cyclic electron flow occurs. It may have formed NPQ. This NPQ-formation rate was slower, but after reaching maximum, NPQ remained at high levels. Although WWC in the *rbc*S antisense plants was lower, these plants always had higher NPQ because of higher ratio of ϕPS I/ϕ PS II irrespective of O_2 concentration. The results indicate that WWC function as a starter of photosynthesis. When WWC is suppressed, the cyclic electron flow can be a second starter. The WWC does not act to maintain a high NPQ and the down-regulation of the quantum yield of PS II is done by the cyclic electron flow.

593 Session 61, Photosynthesis

Photosynthetic adjustments in two rice ecotypes to high growth CO_2 and temperature. WEB SITE: http://www.morris.ars.usda.gov Gesch, Russ W. *USDA-Agricultural Research Service, Morris, MN 56267 USA* Kang, Il-Ho Gallo-Meagher, Maria Boote, Kenneth J. *Agronomy Department, University of Florida, Gainesville, FL 32611 USA* Vu, Joseph C.V. Allen, Jr., L. H. *USDA-Agricultural Research Service, Gainesville, FL 32611 USA* Bowes, George *Botany Department, University of Florida, Gainesville, FL 32611 USA Presenter: Gesch, Russ W. gesch@morris.ars.usda.gov*

Rice (*Oryza sativa* L.) photosynthetically acclimates to elevated CO_2 and is sensitive to high temperatures. However, little is known about intra-specific responses to elevated CO_2 and temperature, although genetic modifications will likely be needed to cope with global climate changes. This study compared adjustments in the photosynthetic apparatus of two rice ecotypes to elevated CO_2 and temperature. IR72 (Indica) and M103 (Japonica) rice were paddy cultured in sun-lit, environment-controlled chambers under ambient (350 ppm) and elevated (700 ppm) CO_2 and day/night temperatures ranging from 28/18 to 40/30°C. At elevated CO_2, leaf photosynthesis in M103 declined with temperature while that of IR72 increased from 28 to 34°C before decreasing at 40°C. At ambient CO_2, the rates were lower for both cultivars with little response to temperature. Rubisco activity and protein content declined with elevated CO_2 and temperature, but the response was greater for M103. Rubisco protein decreased as much as 50% in M103 due to high CO_2. Between 28 and 40°C, Rubisco protein in IR72 leaves declined 20 and 24% under ambient and elevated CO_2, respectively, while it was reduced by 47 and 69%, respectively, in M103. Under ambient CO_2, *rbcS* expression increased in IR72 with temperature, while it decreased for M103 under both CO_2 treatments. Measurements of F_V/F_M showed that neither high temperature or CO_2 affected the photochemical efficiency of PSII. There was a small but consistent increase in *psbA* (codes for D1 protein of PSII) expression for IR72 with temperature, whereas M103 declined slightly. Genotypic variation appears to exists in rice for carboxylation responses to elevated CO_2 and temperature.

594 Session 61, Photosynthesis

Global time- and wavelength-resolution of excitation energy flow and the cold-hard-band in winter-acclimated evergreen leaves at 77K. Gilmore, Adam M Matsubara, Shizue Barker, David H Ball, Marilyn C *Australian National University, Research School of Biological Sciences, Ecosystem Dynamics, Canberra ACT 0200, Australia* Itoh, Shigeru *University of Nagoya Physics Department, Photobioenergetics Group, Chikusa-Ku Nagoya 464–01, Japan Presenter: Gilmore, Adam M gilmore@rsbs.anu.edu.au*

This 77K study resolves the excitation energy flow in the photosynthetic apparatus in two winter-acclimated evergreen species, namely, the snow gum *Eucalyptus pauciflora* Sieb. ex Spreng. and the mistletoe *Amyema miquelii* (Lehm. ex Miq.) Tiegh.. The winter acclimation was associated with dramatically altered spectral features of the fluorescence emitted from the chlorophylls of the photosynthetic apparatus [Gilmore and Ball (2000) 97:11098–111101]. The main feature, termed the cold-hard-band (CHB), covered a broad spectral range (~25 nm) and was centered around 715–720 nm. Example leaves with high and low photosystem II (PSII) photochemical efficiency and inversely varying CHB amplitudes were compared for each species. In leaves with low CHB, the rise-decay kinetics indicate energy is transferred from the 680 nm band of the antenna to the 685 nm band of the PSII core-inner antennae and ultimately to the main photosystem I (PSI) antenna band (740 nm). Leaves with high CHB amplitudes exhibit the same decay times for the F740 band (~2800 ps) as leaves without the CHB. The CHB rise kinetics indicate it receives energy from the antennae and most closely parallels the 685 nm band; the average of the bimodal CHB decay time is around 1500 ps in both species. In leaves with high CHB the fluorescence lifetimes for both the PSII 685 and 695 bands are clearly decreased. The 695 nm band exhibited a rapid rise/decay phase paralleling that of F680 and possibly correlating with energy transfer to the CHB and PSI in all samples. We conclude the spectral-kinetic features associated with winter high-light acclimation including the CHB and PSII energy dissipation are generally conserved among evolutionarily divergent species.

595 Session 61, Photosynthesis

Spatio-temporal heterogeneity of CAM-photosynthesis is closely linked to the metabolic properties of CAM and to tightly packed mesophyll cells. Rascher, Uwe *Biosphere 2 Center, Columbia University* Duarte, Heitor Huett, Marc-Thorsten *Institute of Botany, Darmstadt University of Technology, Germany Presenter: Rascher, Uwe urascher@bio2.columbia.edu*

Spatio-temporal heterogeneity of photosynthetic efficiency governs the subsequent phases of crassulacean acid metabolism (CAM). Thus far this phenomenon was only described for the obligatory CAM-plant Kalanchoë daigremontiana. In this communication we show that spatial variations of effective quantum yield, measured by fluoresecence imaging techniques, are even more pronounced on leaves of the facultative CAM-Plant Clusia minor. However, heterogeneity only emerges during CAM and disappears during C3 photosynthesis, thus, these spatio-temporal variations can be assumed to be a product of the constrains of CAM. Both plants investigated have only comparably little intercellular air-filled space, tightly packed mesophyll cells limit CO2 diffusion inside the leave tissue and isolate adjecent leaf areas. Different leaf anatomy qualitatively influences the type of observed patterns, however, the variations are not related to leaf anatomy in a simple linear way. Correlation analyses and mathematical quantification methods, which are based on cellular automaton techniques, help separating anatomically based form physiologically based heterogeneity of photosynthetic efficiency.