Tropentag, October 7-9, 2008, Hohenheim

"Competition for Resources in a Changing World:
New Drive for Rural Development"

Physiological Adjustment Mechanism of *Medicago truncatula* N_2 Fixation under Environmental Perturbations

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Abstract

To regulate the uptake of nutrients that may be available in excess, plants may use feedback systems. These systems are envisaged to involve the cycling of nutrients within an organ or a plant and the regulation of further nutrient uptake by the products of assimilation. The concept of feedback regulation of N_2 fixation has been developed in the last decade of the previous century as a general mechanism governing regulation of N_2 fixation by environmental factors. Currently, we are testing the validity of such hypothesis on forage legumes under various environmental perturbations using the model plant *Medicago truncatula* as a test crop.

Two experiments were carried in a growth chamber in a nutrient solution and inoculated with *Sinorhizobium meliloti* 102F51. In the 1st experiment, 60 % of the leaves (lower part) were individually darkened (against control) while keeping the percentage of treated leaves constant by further darkening appropriate to new leaf expansion for 2 week. In the 2nd experiment, an open-flow gas measurement system was used to measure H_2 & CO_2 evolution and to calculate N_2 fixation and electron allocation from apparent nitrogenase activity (ANA) [80 % N_2/20 % O_2] and total nitrogenase activity (TNA) [80 % Ar/20 % O_2] before and after high KNO_3 application.

Darkened leaves were senescened and %N in the whole plant was highly increased versus untreated controls thus leading towards the tendency of reduced C/N ratios. Surprisingly, the growth rate of treated plants exceeded untreated controls. The gas measurement trial revealed that H_2 evolution was decreased slightly while electron allocation coefficient (EAC = 1 – ANA/TNA) was increased (11 %) and the amount of N-fixed per day remained stable.

According to the N-feedback hypothesis, excess and high soluble N levels in the shoots reduce N_2 fixation rates after sensing and sending certain signal(s) and the repetition of this messenger(s) can induce nodule senescence. To avoid such detrimental effect the legumes must 'lock up' excess-N in proteins and retain it in this form. This could explain why N_2 fixing legumes are normally protein-rich plants. Additionally, perennial pasture legumes posses other physiological adaptation for such purpose (i.e. tannins and/or polyphenol oxidase).

Keywords: Combined-Nitrogen, leaf darkening, legume, N-feedback, N_2 fixation, regulation

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