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Photosynthetic adaptation of soybean due to varying effectiveness of N_2 fixation by two distinct *Bradyrhizobium japonicum* strains

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ABSTRACT

Rhizobial N_2 fixation is a costly biochemical process, which takes 6–14% of current photosynthate (C) from legumes, without compromising grain productivity. In addition to the effects of leaf N nutrition, rhizobial symbiosis could stimulate photosynthesis due to the removal of C sink limitation by nodule activity. To test that hypothesis, we compared the photosynthetic capacity of soybean plants inoculated with two different strains of *Bradyrhizobium japonicum* (CPAC 390 or CPAC 7), varying in the effectiveness to fix N_2, with plants fertilized with NO_3^-. Nodulated plants had 14–31% higher rates of photosynthesis and accumulated less starch in the leaves than N-fertilized plants. There was evidence that *B. japonicum* CPAC 390 had higher carbon costs of N_2 fixation compared with CPAC 7, but the increases in carbon costs were accompanied by higher rates of photosynthesis. By applying a biochemical model of leaf photosynthesis, including the limitations of Rubisco activity ($V_{C_{max}}$), electron transport rates (J) and triose-P utilization (TPU), we show that soybean plants adapt their photosynthetic capacity to support the stronger carbon sink created by faster rates of N_2 fixation. We observed that plants associated with CPAC 7 (of low effectiveness to fix N_2) increased their photosynthesis by removing sink limitation solely (with a constant $V_{C_{max}}$) whereas plants associated with CPAC 390 (of high effectiveness to fix N_2) increased their photosynthesis by sink stimulation. Based on the model, we propose that sink stimulation is governed by a positive feedback between TPU and Rubisco activation, resulting in an increased $V_{C_{max}}$.

1. Introduction

Legume plants have evolved into the symbiosis with diazotrophic rhizobial bacteria, in which plants exchange photosynthates (C) for N fixed by bacteria from the atmosphere. Although the symbiosis is very beneficial to the plant under low N soil conditions, the C costs of symbiotic N_2 fixation are often assumed to decrease potential legume productivity (Kiers and Denison, 2008). Several studies demonstrated that legumes would spend larger amounts of photosynthates to acquire N through symbiotic N_2 fixation than through absorbing NO_3^- directly from soil solution (Kaschuk et al., 2009). However, despite these arguments, there is little evidence that reducing C costs of N acquisition results in larger plant productivity (Skøt et al., 1986, Kaschuk et al., 2010b). In fact, despite the larger "C costs", legume plants inoculated with effective rhizobial strains, and managed under low N soil conditions, produce more biomass than when they are fertilized with soluble N only (Kaschuk et al., 2010b).

The "C costs" are probably covered by compensatory photosynthesis as photosynthesis is co-stimulated by leaf N nutrition and C sink strength (Stitt and Krapp, 1999; Kaschuk et al., 2009). Indeed, several studies postulated that photosynthesis increases because the "C costs" of nodule activity and N_2 fixation remove the C sink limitation of photosynthesis (Harris et al., 1985). Photosynthesis of rhizobial plants is increased regardless of a lower leaf N concentration in relation to N-fertilized plants (Brown and Bethlenfalvay, 1987; Kaschuk et al., 2010a).

This study tested the hypothesis that rhizobial symbiosis stimulates photosynthesis over and above the expected effect of biological N_2 fixation on leaf N nutrition due to C sink stimulation by nodule activity. To this end, we compared the photosynthetic capacity of plants nodulated with two rhizobial strains, varying

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in the effectiveness of N_2 fixation and putative different C costs, with plants receiving adequate amounts of N fertilizer. The C sink limitation (or stimulation) was checked through measurements of CO_2 response curves of photosynthesis, fitted to a widely used biochemical model including three physiological limitation processes of photosynthesis: Rubisco (ribulose 1,5 biphosphate carboxylase/oxygenase, E.C. 4.1.1.39) activity, electron transport rates (Farquhar et al., 1980) and triose phosphate utilization (TPU; i.e., C sink limitation) (Sharkey, 1985). This approach would allow testing whether nodule activity can remove TPU limitation of photosynthesis.

2. Materials and methods

2.1. Experiment setup

Soybean (*Glycine max* (L.) Merrill; cv. BRS 154, of determinate growth) was inoculated with two *Bradyrhizobium japonicum* strains or fertilized with a nutrient solution containing KNO_3. Two *B. japonicum* strains were chosen: CPAC 7 and CPAC 390, both belonging to the serogroup of *B. japonicum* CB 1809 = SEMIA 586. In a previous study, inoculation of soybean with *B. japonicum* CPAC 390 lead to a significant increase in nodule biomass and total N accumulated, but resulted in a decrease in shoot biomass in comparison with CPAC 7 (Santos et al., 1999). These results could be attributed to a higher effectiveness of N_2 fixation associated with higher C costs by CPAC 390.

Plants were cultivated in 2.5 kg capacity plastic pots filled with a mixture of sterilized sand and vermiculite (1:1). Seeds were surface sterilized according to Vincent (1970) before sowing. All plants received sterilized N-free solution (Broughton and Dilworth, 1971) with the pH adjusted to 6.8. Development stages were defined according to Fehr et al. (1971). The non-inoculated plants received a KNO_3 20.8 mM nutrient solution three times at vegetative stage (emergence, V4 and V5) and one time at reproductive stage (R1), in total, an amount of 210 mg N per plant, to prevent nodulation by contaminant rhizobia. Rhizobia were grown in yeast mannitol (YM) medium according to Vincent (1970) up to the density of 10^9 cells mL^{-1}; and 1 mL of the suspension was added per seed of the inoculated treatments at sowing. Seven seeds were sown in each pot, and plants were thinned to one plant per pot at V1 stage (5 d). We started measuring leaf gas exchange at stage R2 (35 d) and finished measurements before the stage R4 (40 d). Maximum photosynthetic active radiation (PAR) in the glasshouse averaged 600 μmol quanta m^{-2} s^{-1} during the growth period. Temperatures in the glasshouse during the period of the experiment averaged 33/22 °C (day/night).

2.2. Gas exchange and chlorophyll fluorescence measurements

The measurements of responses of photosynthesis (A) to increasing intercellular CO_2 concentrations (C_i) – A/C_i curves – were performed on the third expanded leaf (three replicates in each treatment), using the open gas exchange system Li-6400 (LI-COR Inc., Lincoln, NE, USA). Measurements started around 11:00 h and stopped not later than 16:30 h. A full response curve took 50 min–1 h to be completed. During the measurements, the air temperature in the glasshouse varied from 33 to 36 °C. An area of 2 cm^2 of leaf was enclosed in a broadleaf chamber (6 cm^2), which received a steady flow rate of 500 μmol air s^{-1} with different air CO_2 concentrations (C_a, μmol CO_2 mol^{-1} air) for each step. The first step consisted of 20 min of dark adaptation, and C_a of 350 μmol mol^{-1} to ensure a steady-state activation of Rubisco (Long and Bernacchi, 2003). The second step consisted of re-adaptation of leaf to actinic light for 0.1 min. Then, the C_a was decreased to 50 μmol mol^{-1}, and then increased progressively to 100, 150, 200, 250, 350, 500, 650, 1000, 1500 and 2000 μmol mol^{-1}. The different C_a was obtained automatically with a CO_2 injector System (Li-cor 6400-01), which mixed CO_2-free air and high pressure pure liquefied CO_2. The leaf chamber ambient air composition was adjusted to maintain steady-state of ambient O_2 concentration (210 mmol mol^{-1}), PAR of 1000 μmol quanta m^{-2} s^{-1} and leaf temperature of 32 °C. The leaf-to-air vapour pressure difference varied from 1.5 to 3.0 kPa. All CO_2 exchange data were corrected for leakage of CO_2 into and out the leaf cuvette, using thermally killed leaves according to Flexas et al. (2007).

2.3. Shoot sampling

The leaves used for photosynthesis measurements were labelled and, when all the measurements of CO_2 response curves were finished (stage R3/R4), were harvested in the afternoon (16.30 h), frozen in liquid nitrogen and stored at −80 °C for sugar and starch analyses. The remaining shoots were dried at 60 °C for 48 h and weighed. Leaves were ground for total N. Roots and nodules were thoroughly but gently washed with tap water and dried at 60 °C for 48 h. After that, nodules were detached, counted and weighed.

2.4. Leaf chemical analyses

Soluble sugars and starch were extracted by ethanol (Hungria, 1994). The soluble sugars (hexoses and their methylated derivatives) were determined based on Dubois et al. (1956). The total starch was analyzed according to the enzymatic method of McClearly et al. (1997) using a commercial assay kit (K-TSTA, Megazyme International Ireland Ltd., Bray, Republic of Ireland). Total N was extracted from 100 mg of dry ground leaves with a sulfuric acid digestion according to the Kjeldahl method. The obtained leaf N was then converted to leaf N content (g N m^{-2}) using specific leaf area. Chlorophyll content was determined with the chlorophyllmeter SPAD 502 (Konica Minolta Sensing Inc., Osaka, Japan) adjusted according to preliminary calibration (Kaschuk et al., 2010a).

2.5. Biochemical model of leaf photosynthesis

We used the model of Farquhar et al. (1980), later modified by Sharkey (1985), for C_3 photosynthesis to analyze photosynthetic regulation of our experimental plants. The model assumes that A (μmol CO_2 m^{-2} s^{-1}) is determined by three limiting processes: carboxylation limited by Rubisco activity (A_C), by electron transport (A_J), and by TPU (A_P) according to the equation:

$$A = \min(A_C, A_J, A_P)$$

For simplicity of our model analysis we assumed no significant mesophyll resistance. With this assumption, the first two limiting processes [A_C, A_J (based on the NADPH demand)] can be described as follows:

$$A_C = \frac{V_{C_{max}} \cdot (C_i - \Gamma^*)}{C_i + K_{mC}(1 + O_i/K_{mO})} - R_d$$

where $V_{C_{max}}$ is the maximum rate of Rubisco carboxylation, R_d is the mitochondrial respiration in the light (μmol CO_2 m^{-2} s^{-1}), assumed to be directly related to $V_{C_{max}}$ at 25 °C (Watanabe et al., 1994) as: $R_d = 0.0089 \cdot V_{C_{max}}$; and, Γ^* is the CO_2 compensation point in the absence of R_d, estimated as $\Gamma^* = 0.5 \cdot O_i \cdot (K_{mC}/K_{mO})(V_{O_{max}}/V_{C_{max}})$ (Farquhar et al., 1980).

$$A_J = J \cdot \frac{C_i - \Gamma^*}{4 \cdot C_i + 8 \cdot \Gamma^*} - R_d$$

Table 1
Parameters used in the photosynthesis model of this study.

Symbol	Description	Input value	Reference
$K_{mC\,25}$	Michaelis–Menten constant for CO_2 at 25 °C	405 μmol mol^{-1}	Bernacchi et al. (2001)
$K_{mO\,25}$	Michaelis–Menten constant for O_2 at 25 °C	278 mmol mol^{-1}	Bernacchi et al. (2001)
$E_{K_{mC}}$	Activation energy for K_{mC}	79,430 J mol^{-1}	Bernacchi et al. (2001)
$E_{K_{mO}}$	Activation energy for K_{mO}	36,380 J mol^{-1}	Bernacchi et al. (2001)
$E_{V_{Cmax}}$	Activation energy for V_{Cmax}	65,330 J mol^{-1}	Bernacchi et al. (2001)
E_J	Activation energy for J	37,000 J mol^{-1}	Farquhar et al. (1980)
E_{T_P}	Activation energy for T_P	53,100 J mol^{-1}	Harley et al. (1992)
E_{R_d}	Activation energy for R_d	46,390 J mol^{-1}	Bernacchi et al. (2001)
D_J	Deactivation energy for J	200,000 J mol^{-1}	Medlyn et al. (2002)
D_{T_P}	Deactivation energy for T_P	201,800 J mol^{-1}	Harley et al. (1992)
S_J	Entropy term for J	650 J K^{-1} mol^{-1}	Harley et al. (1992)
S_{T_P}	Entropy term for T_P	650 J K^{-1} mol^{-1}	Harley et al. (1992)
R	Universal gas constant	8.314 J K^{-1} mol^{-1}	Farquhar et al. (1980)

where J is the rate of linear electron transport at the light level of measurement (i.e., 1000 μmol m^{-2} s^{-1} in our case).

Net CO_2 assimilation, determined by the third limiting process, is simply:

$$A_P = 3 \cdot \mathrm{TPU} - R_d$$

where TPU is the rate of triose phosphate export. It is multiplied by 3 because 3 mol of CO_2 can be fixed for every mol of triose-P made available.

To account for small fluctuations in leaf temperature during measurements, we included temperature response functions in the model analysis. The temperature dependent kinetics for the calculation of $V_{C_{max}}$, K_{mC}, K_{mO} and R_d was described by an Arrhenius function normalized with respect to 25 °C (von Caemmerer, 2000):

$$\mathrm{parameter}(T) = \mathrm{parameter}(25\,^\circ\mathrm{C})e^{[(T_l-25)E_{\mathrm{parameter}}/(298R(273+T_l))]}$$

where parameter can be $V_{C_{max}}$, R_d, K_{mC} or K_{mO}; T_l is the leaf temperature (°C); E is the activation energy and R is the universal gas constant. The temperature dependent kinetics for the calculation of J and T_P with respect to 25 °C was described by Medlyn et al. (2002):

$$\mathrm{parameter}(T) = \mathrm{parameter}(25\,^\circ\mathrm{C}) \cdot e^{(E_{\mathrm{parameter}}(T_l-25)/298R(273+T_l))} \cdot \frac{1+e^{(298S_{\mathrm{parameter}}-D_{\mathrm{parameter}})/(298R)}}{1+e^{[(T_l+273)S_{\mathrm{parameter}}-D_{\mathrm{parameter}}]/R(T_l+273)}}$$

where parameter is J or T_P, S is the entropy term, E and D are the energies of activation and deactivation, respectively. Input values for parameters related to the above temperature response are given in Table 1, mainly based on Bernacchi et al. (2001), who also assumed a negligible mesophyll-diffusion resistance of CO_2 transfer. Similarly, parameters for the temperature response of the $V_{O_{max}}/V_{C_{max}}$ ratio, required for calculating Γ^*, were based on Bernacchi et al. (2001).

To predict A for the nodulated plants using parameters estimated for the N-fertilized plants, the effects of different leaf N contents in these plants on the $V_{C_{max}}$ and J were accounted for by applying a linear relationship:

$$V_{C_{max}} = V_{C_{max(fert)}} + 60.0[\mathrm{N}_{(nod)} - \mathrm{N}_{(fert)}]$$

$$J = J_{(fert)} + 98.1[\mathrm{N}_{(nod)} - \mathrm{N}_{(fert)}]$$

where $\mathrm{N}_{(nod)}$ and $\mathrm{N}_{(fert)}$ are the leaf N content (g N m^{-2} leaf) in nodulated and N-fertilized plants, respectively. The coefficients 60.0 and 98.1 were obtained from the observations by Harley et al. (1992), since it has been shown that they were quite conservative within certain range of leaf N values. The adjustment of leaf N is needed to circumvent biased effects of leaf N status.

2.6. Statistical analyses

Data were tested for homogeneity with Levene's test of equality of error variances and normality was checked for normal distributions, and then submitted to one-way ANOVA with SPSS 15.0.1 for windows (SPSS Inc., 1989–2006). Tukey's test was employed as a post-hoc multiple range test. The model-fitting of the A vs. C_i response curves was programmed with the non-linear least-squares regression of the Gauss method in the PROC NLIN of the SAS 9.1.3 Software for Windows (SAS Institute Inc., Cary, NC, USA). The input values used are presented in Table 1.

3. Results

3.1. Photosynthesis and leaf chemical analyses

Net rates of photosynthesis at saturated CO_2 concentrations were 35 μmol m^{-2} s^{-1} and 28 μmol m^{-2} s^{-1} in plants nodulated with *B. japonicum* CPAC 390 and CPAC 7, respectively, which was 31 and 14% more than in N-fertilized plants. At 350 μmol mol^{-1} CO_2, net photosynthesis of plants nodulated with *B. japonicum* CPAC 390 was 23% higher than in plants nodulated with *B. japonicum* CPAC 7 or fertilized with N (data not shown). Plants of the three treatments grew well under glasshouse conditions, and despite differences in leaf photosynthesis, all treatments produced similar amounts of shoot biomass (Table 2). Inoculated plants supported adequate nodule biomass at stage R2 regardless of *Bradyrhizobium* strain, whereas N-fertilized plants were void of nodules. Differences in photosynthesis could not be explained by leaf N concentration. Leaf N concentration and chlorophyll concentration did not differ significantly between treatments (Table 2).

Higher rates of photosynthesis at saturated CO_2 concentrations were associated with lower accumulation of starch in the leaves. N-fertilized plants accumulated 1.9 and 2.4 times more starch than plants nodulated with CPAC 7 and CPAC 390, respectively (Table 2). Soluble sugars did not differ between the three treatments. As a result, the starch to sugar ratio decreased with increases in photosynthesis at saturated CO_2 concentrations. N-fertilized plants had the lowest rates of photosynthesis and the highest starch to sugar ratio, whereas plants nodulated with CPAC 390 had the highest rates of photosynthesis and the lowest starch to sugar ratio. Higher rates of photosynthesis were associated with higher N:starch ratio. Plants nodulated with CPAC 390 had significantly higher rates of

Table 2
Shoot and nodule dry weight, contents of N, chlorophyll, starch and sugar in the leaves of soybeans (cv. BRS 154) inoculated with two *Bradyrhizobium japonicum* strains (CPAC 7 or CPAC 390) or fertilized with N. Mean ± standard deviation; $n = 4$ for shoot, $n = 3$, otherwise.

Parameter	CPAC 390	CPAC 7	N-fertilized	*P*-value
Shoot[a] (g plant^{-1})	2.4 ± 1.0	2.7 ± 0.3	2.5 ± 0.1	ns
Nodule (g plant^{-1})	0.5 ± 0.1	0.6 ± 0.0	0.0	
Leaf N (mg N g^{-1})	36.2 ± 1.4	32.3 ± 6.7	29.9 ± 10.4	ns
Chlorophyll (g m^{-2})	142.6 ± 1.0	153.5 ± 11.8	154.1 ± 9.5	ns
Starch (mg g^{-1})	32.8 ± 16.7b	42.3 ± 12.7 b	79.9 ± 7.5a	0.012
Sugar (mg g^{-1})	48.6 ± 5.3	43.5 ± 16.2	48.6 ± 5.3	ns
Starch: Sugar ratio	0.7 ± 0.3b	1.0 ± 0.2ab	1.6 ± 0.4a	0.023
N: starch ratio	1.3 ± 0.5a	0.8 ± 0.2ab	0.4 ± 0.1b	0.041

[a] Shoot biomass was measured in a second trial, repeated under the same conditions up to the same developmental stage. Different letters indicate differences at $P > 0.05$ by the Tukey test.

photosynthesis and N:starch ratios than N-fertilized plants. Plants nodulated with CPAC 7 had intermediate rates of photosynthesis and also an intermediate N:starch ratio in the leaves (Table 2).

3.2. Model analysis of leaf photosynthesis

Application of the full biochemical model of leaf photosynthesis to *A* vs. C_i response curves allowed us to estimate the three parameters: *J* (μmol e$^-$ m^{-2} s^{-1}), $V_{C_{max}}$ (μmol CO_2 m^{-2} s^{-1}) and TPU (μmol triose-P m^{-2} s^{-1}). Plants nodulated with CPAC 390 had significantly higher values of TPU and $V_{C_{max}}$ than plants nodulated with CPAC 7 or fertilized with N (Fig. 1). There was more variability in the estimated *J* of N-fertilized plants than of nodulated plants and therefore, *J* in N-fertilized plants was similar to *J* of plants nodulated with CPAC 7. The estimated *J* of plants nodulated with CPAC 390 was significantly higher than of plants nodulated with CPAC 7.

To test the hypotheses on sink limitation removal vs. direct sink stimulation of photosynthesis, we parameterized the full model of leaf photosynthesis with the N-fertilized plants, and then used these parameter estimates to predict the rates of photosynthesis of nodulated plants, by excluding the photosynthesis limitation of TPU (reduced model). Parameterization of the full model of leaf photosynthesis considering Rubisco, electron transport and TPU limitations for the N-fertilized plants is shown in Fig. 2, together with the concentration of intercellular CO_2 at which a transition from one limitation to another is expected. The best least-squares estimates of the parameter values were: $V_{C_{max}} = 59.4$ μmol CO_2 m^{-2} s^{-1}, $J = 140.9$ μmol e$^-$ m^{-2} s^{-1} and TPU = 6.3 μmol triose-P m^{-2} s^{-1}. Before applying the reduced model to predict photosynthesis of nodulated plants, the estimates of *J* and $V_{C_{max}}$ were corrected for the small differences in leaf N content between nodulated and fertilized plants.

The reduced model predicted the rates of photosynthesis in plants nodulated with CPAC 7 well, but strongly underestimated the rates of photosynthesis in plants nodulated with CPAC 390 (Fig. 3). The higher measured rates of photosynthesis of plants

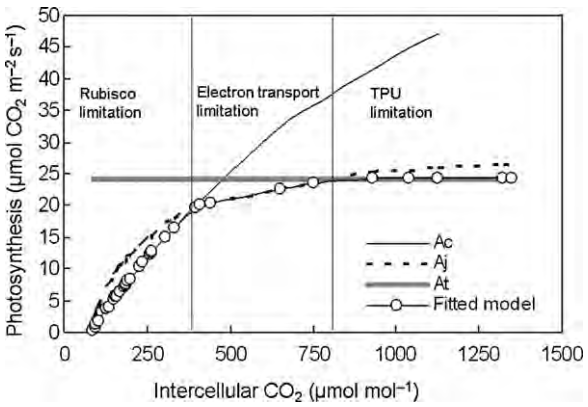

Fig. 2. Model fit to A/C_i response curves measured on leaves of N-fertilized soybean plants. Best least-squares estimates of the parameter values were: $V_{C_{max}}$ = 59.4 μmol CO_2 m^{-2} s^{-1}, $J = 140.9$ μmol e$^-$ m^{-2} s^{-1}, TPU = 6.3 μmol triose-P m^{-2} s^{-1}.

nodulated with CPAC 390 than predicted by removal of sink (TPU) limitation suggest that photosynthesis was directly sink stimulated. The linear regression between predicted and measured rates of photosynthesis for plants nodulated with CPAC 390 had larger intercept and slope values than those estimated for plants nodulated with CPAC 7 (Fig. 4). Since the intercept of the regression gives an indication of agreement between measured and predicted *A* at the low C_i range, the large intercept value for the CPAC 390 treatment suggests that photosynthesis was already stimulated in plants nodulated with CPAC 390 well before the TPU limitation, which is expected to occur at saturated intercellular CO_2 concentrations.

4. Discussion

The "C costs" of N_2 fixation, which is mainly determined by the presence and efficiency of some enzymes in the bacteroids and nodules, has puzzled agronomists with respect to legume productivity

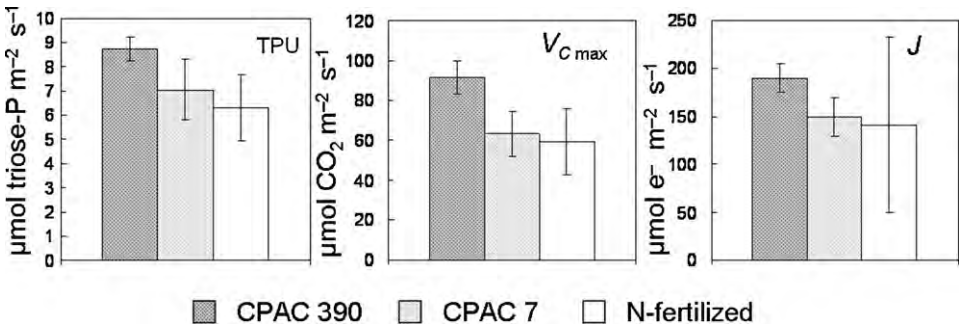

Fig. 1. Parameters TPU, $V_{C_{max}}$ and *J*, obtained by curve-fitting of A/C_i response curves of photosynthesis in leaves of soybeans inoculated with *Bradyrhizobium japonicum* strains (CPAC 7 or CPAC 390) or fertilized with N. Bars indicate 95% confidence intervals.

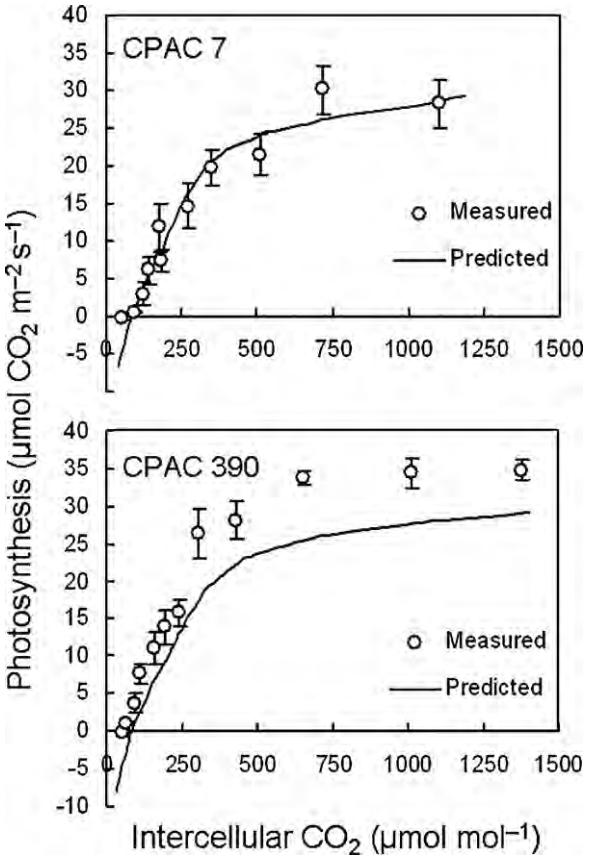

Fig. 3. Measured and predicted photosynthetic response curves with increasing intercellular CO_2 concentrations of leaves of soybeans inoculated with *Bradyrhizobium japonicum* strains (CPAC 7 or CPAC 390). Predicted values were obtained by the model parameterized with the A/C_i response curves of N-fertilized plants (Fig. 1), but excluding the TPU limitation and correcting for increased leaf N concentration.

under symbiotic conditions. Phillips (1980) suggested that legume growth could be improved by increasing the energy efficiency of N_2 fixation, in other words by reducing the relative costs in terms of g C g N^{-1}; thus a rhizobial strain with less C costs of N_2 fixation would be preferred for use in inoculants. However, Skøt et al. (1986) observed that strains of higher effectiveness had larger C costs of N_2 fixation. Nodulated plants also produce more biomass than N-fertilized plants (Kaschuk et al., 2010b). In this study, we observed

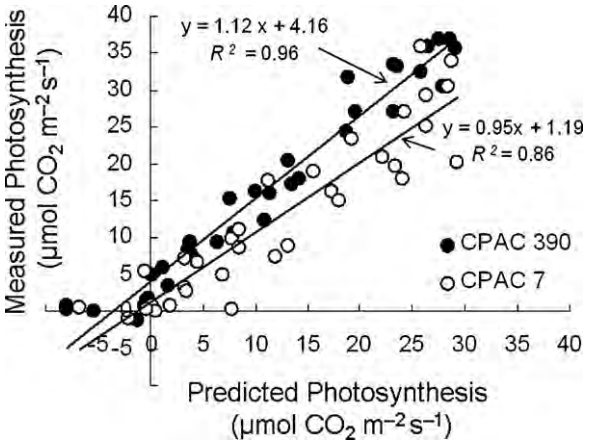

Fig. 4. Linear regression between measured and predicted rates of photosynthesis in leaves of soybeans inoculated with *Bradyrhizobium japonicum* strains (CPAC 7 or CPAC 390).

plants nodulated with CPAC 390 strain had higher N:starch ratio in the leaves than plants nodulated with CPAC 7 (Table 2), indicating that for each N fixed, less starch was accumulated in the leaves. Furthermore, the application of the full model of leaf photosynthesis predicted that plants nodulated with CPAC 390 had higher rates of TPU – meaning higher photosynthate export from chloroplast – than those plants inoculated with CPAC 7 or fertilized with N (Fig. 1). We surmise that differences in TPU between plants nodulated with the two strains were due to different C costs of N_2 fixation.

In this study, plants nodulated with CPAC 390 had much high rates of photosynthesis than those nodulated with CPAC 7, which shows that soybean plants can compensate for the increased C costs for N_2 fixation by increasing photosynthesis due to sink stimulation (e.g., Harris et al., 1985; Ainsworth et al., 2004; Kaschuk et al., 2009, 2010a,b). The model fitted into actual photosynthesis rates predicted differences in $V_{C_{max}}$ and TPU of plants nodulated with CPAC 390 and CPAC7, and no differences in $V_{C_{max}}$ and TPU of plants nodulated with CPAC 7 and fertilized with N. In the comparison of the two rhizobial strains, there is an indication that increased rates of TPU lead to a positive feedback on the rates of $V_{C_{max}}$. In fact, one of the first products of photosynthesis is triose-P, which can be converted to starch or stored temporally in the chloroplast depending on the C sink strength of the plants. Provided that P supply is not a major constraint (de Groot et al., 2003), plants with a stronger C sink from nodule activity tend to accumulate less starch in the leaves (Huber and Israel, 1982; Kaschuk et al., 2010a) and unload more sucrose into the phloem, which in turn, accelerates the exchange rate of triose phosphate per orthophosphate and triggers the enzymes related to CO_2 fixation (Azcón-Bieto, 1983; Kaschuk et al., 2009).

Furthermore, the model predicted no differences in J between plants nodulated with any of the rhizobial strains or fertilized with N. Therefore, J was not an adequate parameter to compare the photosynthetic capacity between different treatments. In fact, plants depending on NO_3^- reduction may sustain high rates of electron transport because, apart from the demand for reductants to support CO_2 fixation, N-fertilized plants utilize reductants to reduce NO_3^- (e.g., Cen and Layzell, 2003; Yin et al., 2006).

Model simulations suggested that the removal of sink limitation of photosynthesis explained the increases in the plants nodulated with CPAC 7 (Fig. 3); so the difference between simulated and observed photosynthesis converged just by removing sink (TPU) limitation (Fig. 3). By contrast, removal of sink limitation solely did not explain the increase in photosynthesis in the plants nodulated with CPAC 390 (Fig. 3). The increases in photosynthesis in these plants were explained by direct sink stimulation, through a feedback between TPU and $V_{C_{max}}$. Indeed, Fig. 4 shows that the intercept of the linear regression of predicted and measured rates of photosynthesis on plants nodulated with CPAC 390 occurred before that of plants nodulated with CPAC 7, at levels of C_i well lower than the saturated CO_2 concentration at which the TPU limitation is expected, due to increased $V_{C_{max}}$. It demonstrates that soybean plants adapt their photosynthetic capacity to support different C sink strengths of N_2 fixation. In plants associated with CPAC 7 [of low effectiveness to fix N_2; Santos et al. (1999)], photosynthesis is increased by the removal of sink limitation solely (with a constant $V_{C_{max}}$); however, in plants inoculated with CPAC 390 [of high effectiveness to fix N_2; Santos et al. (1999)], photosynthesis is increased by sink stimulation, which based on the model is explained by a positive feedback between TPU and Rubisco activation, resulting in an increased $V_{C_{max}}$.

The assumption that large C costs of N_2 fixation decrease legume productivity must be reviewed. Soybean adapts its photosynthetic capacity to support increased C sink strength from nodule activity. Increased photosynthesis due to sink stimulation of

mutualistic root symbionts is not limited to legumes in association with rhizobia. Sink stimulation has also been shown for arbuscular mycorrhizal plants (Kaschuk et al., 2009) and ectomycorrhizal plants (Côrrea et al., 2011).

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