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Regulation of Photosynthesis in Nitrogen Deficient Wheat Seedlings

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ABSTRACT

Nitrogen effects on the regulation of photosynthesis in wheat (*Triticum aestivum* L., cv Remia) seedlings were examined. Ribulose 1,5-bisphosphate carboxylase/oxygenase was rapidly extracted and tested for initial activity and for activity after incubation in presence of CO_2 and Mg^{2+}. Freeze clamped leaf segments were extracted for determinations of foliar steady state levels of ribulose 1,5-bisphosphate, triose phosphate, 3-phosphoglycerate, ATP, and ADP. Nitrogen deficient leaves showed increased ATP/ADP and triose phosphate/3-phosphoglycerate ratios suggesting increased assimilatory power. Ribulose 1,5-bisphosphate levels were decreased due to reduced pentose phosphate reductive cycle activity. Nevertheless, photosynthesis appeared to be limited by ribulose 1,5-bisphosphate carboxylase/oxygenase, independent of nitrogen nutrition. Its degree of activation was increased in nitrogen deficient plants and provided for maximum photosynthesis at decreased enzyme protein levels. It is suggested that ribulose 1,5-bisphosphate carboxylase/oxygenase activity is regulated according to the amount of assimilatory power.

The relationship between photosynthesis and nitrogen fertilization has been investigated frequently (5–10, 14, 21, 22, 25). It has been shown that photosynthesis is decreased in nitrogen deficient plants. Analyses of gas exchange at various CO_2 concentrations and light intensities and of amounts of RuBPCO,[1] Chl and photosystem activities suggest that photosynthesis in nitrogen deficient plants is more limited by RuBP carboxylation than by RuBP regeneration. In the present study, nitrogen effect on the regulation of photosynthesis was analyzed. The results suggested that nitrogen deficient plants have increased amounts of products from photochemical reactions (assimilatory power), decreased catalysis of RuBP regeneration from TP, and increased degree of activation of RuBPCO which catalyses the rate limiting step of photosynthesis.

MATERIALS AND METHODS

Growing Conditions. Wheat (*Triticum aestivum* L. cv Remia) was grown from seeds in pots (12 cm diameter) filled with silica sand (0.3–0.8 mm) in a growth cabinet. The photoperiod was 16 h with light being provided by a bank of fluorescent tubes and incandescent bulbs giving a photosynthetic irradiance of 540 $\mu E\ m^{-2}\ s^{-1}$. Temperature was 18/13°C, humidity was 70/85% (day/night). Plants were irrigated with nutrient solution in the morning and water in the evening. The nutrient solution according to Hammer *et al.* (11) was modified using 7 mM KNO_3 and 2.5 mM $Ca(NO_3)_2$ at the highest nitrogen level. NO_3^- was replaced by Cl^- at lower nitrogen levels. Plants were sampled 7, 10, 14, or 17 d after sowing.

Determination of Steady State Levels of Metabolites. Leaves were sampled after 4 to 8 h of light and extracted as described by Leegood and Furbank (15) using freeze-stop tongs. Frozen leaves (about 15 cm^2) were homogenized in liquid N_2 in a mortar and mixed with 1 ml frozen 1 M $HClO_4$. The thawed extract was transferred to a tube and the mortar rinsed with 0.5 ml ice-cold 0.1 M $HClO_4$. The extract was kept on ice for 30 min and then centrifuged at 40,000*g* for 5 min. The supernatant was neutralized with 5M K_2CO_3, divided into several portions, and stored at −20°C for 10 d or less. Proteins were extracted in 0.1 N NaOH from pellets and quantitated by the Bio-Rad method, with BSA as a reference. RuBP was determined in a 20 μl sample which was treated with HCl to remove HCO_3^-. The procedure of Holtum *et al.* (13) was followed, whereby RuBPCO was purified as described earlier (19). $NaH^{14}CO_3$ (0.6 Ci/mol) was added to the sample before RuBPCO. Adenylates were determined by the luciferase method with LKB chemicals, following the procedure of Lundin *et al.* (16). Extracts for PGA and TP determinations were treated with charcoal (10 mg/ml), centrifuged, and then assayed according to Czok (2).

Determination of RuBPCO Activity. Initial activity of RuBPCO was assayed after rapid extraction as described earlier (17). Part of the extract was incubated for 15 min at 30°C in the presence of 10 mM $NaHCO_3$, 20 mM $MgCl_2$, and 5 mM K_2HPO_4 and then assayed. Assay temperature was 10°C to preserve the degree of activation in the assay mixture. Soluble protein was determined by the Bio-Rad method.

RESULTS AND DISCUSSION

Contents of Protein and Activatable RuBPCO. The protein content of the plants was strongly affected by the nitrogen nutrition within our experimental range (Fig. 1). The content of activatable RuBPCO per mg protein did not change with nitrogen nutrition. Therefore, the change in protein content reflected an analogous change in activatable RuBPCO.

ATP/ADP and TP/PGA Steady State Ratios. Nitrogen deficiency led to increased ratios of ATP to ADP and of TP to PGA (Figs. 2 and 3). The effect on the ATP/ADP ratio was evident 7 d after sowing and persisted during the following 10 d (Fig. 2B).

The formation of TP from PGA in the pentose phosphate reductive cycle is associated with the hydrolysis of ATP and with the oxidation of NADPH, as described by Eq. 1:

$$PGA + ATP + NADPH + H^+ \leftrightarrow TP + ADP + Pi + NADP^+ \quad (1)$$

This reaction *in vivo* is not far from the thermodynamic equilib-

[1] Abbreviations: RuBPCO, ribulose 1,5-bisphosphate carboxylase/oxygenase; RuBP, ribulose 1,5-bisphosphate; TP, triose phosphate; PGA, 3-phosphoglycerate; P_A, assimilatory power.

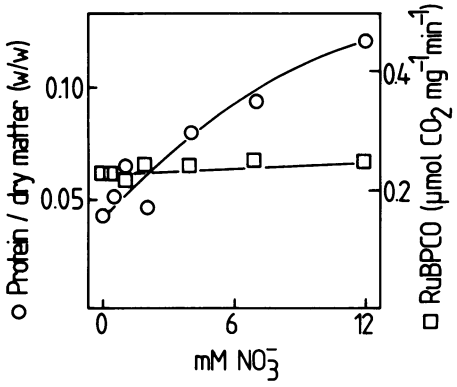

FIG. 1. Effect of NO_3^- concentration in nutrient solution on the contents of soluble protein (weight per dry weight) and activatable RuBPCO (activity at 10°C per mg protein, after incubation in presence of CO_2 and Mg^{2+}) in leaves of 14 d old wheat seedlings.

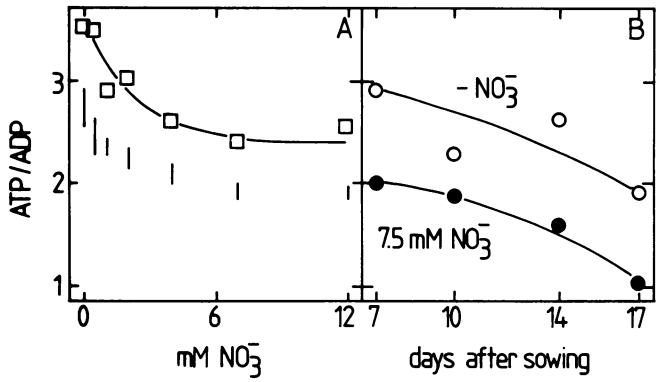

FIG. 2. ATP/ADP steady state ratio (mol/mol) in leaves of wheat seedlings; A, irrigated with nutrient solutions having various NO_3^- concentrations, 14 d after sowing; B, irrigated with 7.5 mM NO_3^- (●) and without NO_3^- (○), on various days after sowing. Means of three determinations are shown. Vertical bars indicate SE.

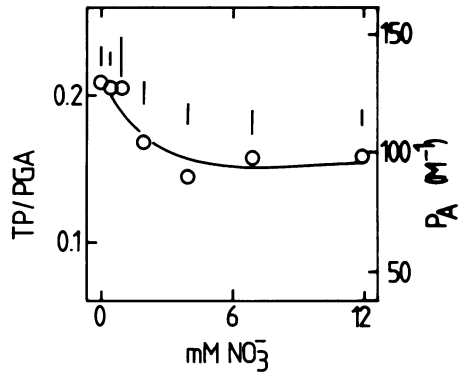

FIG. 3. TP/PGA steady state ratio (mol/mol) in leaves of wheat seedlings, irrigated with nutrient solutions having various NO_3^- concentrations, 14 d after sowing. P_A was calculated from TP and PGA according to Eq. 3 assuming pH 7.8. Means of three determinations are shown. Vertical bars indicate SE.

rium (3). The energetics of PGA reduction is described by Eq. 2:

$$\frac{[PGA][ATP][NADPH][H^+]}{[TP][ADP][Pi][NADP^+]} = 9.8 \times 10^{-6} \quad (2)$$

The phosphorylation potential [ATP]/[ADP][Pi] and redox ratio [NADPH]/[$NADP^+$] are components of P_A. P_A can therefore be calculated from steady state chloroplast levels of TP, PGA, and H^+ (4), (Eq. 3).

$$P_A = \frac{[ATP]}{[ADP][Pi]} \times \frac{[NADPH]}{[NADP^+]} = \frac{[TP]\ 9.8 \times 10^{-6}}{[PGA][H^+]} \quad (3)$$

Extra chloroplast levels of ATP, ADP, TP, and PGA may deviate from chloroplast levels (26) due to a difference in pH. However, extra chloroplast levels are influenced by chloroplast levels due to the TP/PGA shuttle across the chloroplast envelope (12). It is suggested that changes in chloroplast levels induce changes in extrachloroplast levels and are therefore reflected by changes of levels in the whole cells. Hence, the data presented in Figures 2 and 3 indicate an increase in P_A when nitrogen deficiency is induced. P_A in Figure 3 is calculated assuming a pH of 7.8 (4).

The increased TP/PGA ratio in N-deficient plants appears to be due to decreased TP consumption in the pentose phosphate reductive cycle. On the other hand, it could also reflect a decreased flow of TP into the synthesis of organic nitrogen compounds (amino acids) (21).

Regeneration of RuBP. Chl, protein, leaf area, and leaf weight are strongly affected by nitrogen nutrition and are therefore inadequate bases for metabolite levels. The total of adenylates (ATP + ADP + AMP) appears to be more adequate and is used as a basis for comparing RuBP, TP, and PGA levels in Figure 4A. TP per total adenylates was independent of nitrogen nutrition, whereas PGA decreased slightly and RuBP strongly as nitrogen deficiency was induced.

A decrease in RuBP/TP ratio in nitrogen deficient plants is shown more clearly in Figure 4B and suggests that the regeneration of RuBP from TP was increasingly limited as nitrogen nutrition was decreased and that this limitation was not due to a lack of the substrates TP and ATP (Figs. 2 and 3) but rather to insufficient catalysis of enzymic reactions. An increase of the PGA/RuBP ratio in nitrogen deficient plants suggested active RuBP consumption (Fig. 4C). However, it has to be noted that TP and PGA include extra chloroplast components.

RuBP levels did not appear to decrease below binding site concentrations of RuBPCO for RuBP. Plants fertilized with 7.5 mM NO_3^- had 52 nmol RuBP/mg protein and nitrogen deficient plants still had 29.5 nmol/mg protein suggesting that RuBP concentration in nitrogen deficient plants was at least 1.8 times the concentration of binding sites. Photosynthesis appeared therefore to be limited by RuBPCO activity, even in nitrogen deficient plants.

Degree of Activation of RuBPCO. Nitrogen deficiency caused an increase in the degree of activation of RuBPCO (Fig. 5). The effect was already apparent 7 d after sowing and persisted during the following 10 d (Fig. 5B). The increased activation of RuBPCO in nitrogen deficient plants compensated partly for the decrease in the RuBPCO content.

Nitrogen deficiency induced similar effects on assimilatory power and RuBPCO activation, suggesting that RuBPCO is regulated according to the availability of ATP and NADPH from the photochemical reactions. Similar conclusions can be drawn from experiments with Pi deficient chloroplasts (18, 19) and leaves at low temperature and low oxygen (23). Decreased levels of ATP and RuBPCO activity in Pi deficient isolated chloroplasts were associated with preferential export of PGA instead of TP suggesting that PGA was accumulated and stromal pH was decreased (18). Deactivation of RuBPCO was suggested to be due to this decrease in stromal pH (19). Stromal pH in nitrogen deficient leaves could be increased due to decreased PGA level and the increase in RuBPCO activity could be due partly to this increase in pH. The decreased RuBP level could contribute to

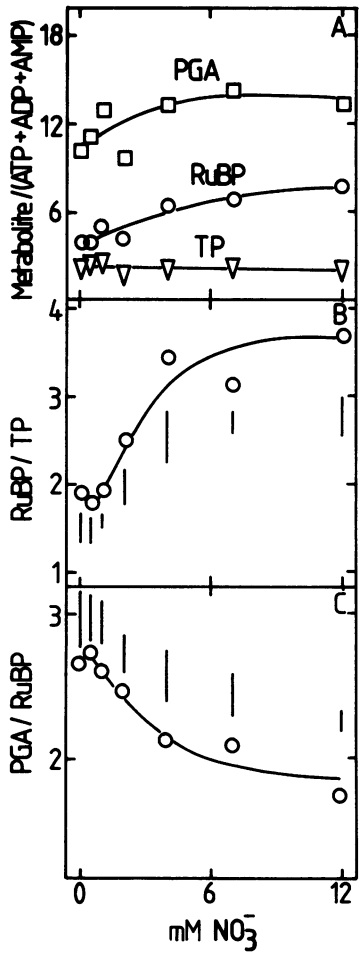

FIG. 4. Molar ratios of steady state metabolite levels in leaves of wheat seedlings, irrigated with nutrient solutions having various NO_3^- concentrations, 14 d after sowing. A, RuBP (○), PGA (□), and TP (▽) per total adenylates; B, RuBP/TP; C, PGA/RuBP. Means of three determinations are shown. Vertical bars indicate SE.

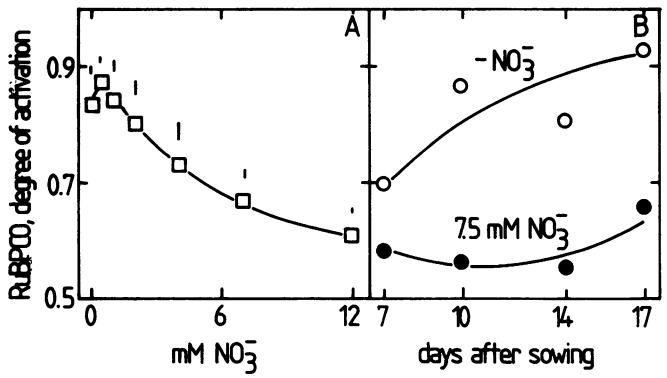

FIG. 5. Degree of activation of RuBPCO (initial activity/activity after incubation in the presence of CO_2 and Mg^{2+}) in leaves of wheat seedlings; A, irrigated with nutrient solutions having various NO_3^- concentrations, 14 d after sowing; B, irrigated with 7.5 mM NO_3^- (●) or without NO_3^- (○), on various days after sowing. Means of three determinations are shown. Vertical bars indicate SE.

this increase in RuBPCO activity in nitrogen deficient leaves, since RuBP is known to inactivate RuBPCO, especially at low pH (20). The decrease in PGA and RuBP levels may result in increased Pi levels which could also contribute to RuBPCO activation in nitrogen deficient plants, since Pi is suggested to be an important activator of RuBPCO in leaves (1). ATP *per se* could also contribute to RuBPCO activation, since ATP is needed for the operation of RuBPCO activase (24). This would suggest a direct effect of assimilatory power on RuBPCO activity.

CONCLUSION

Limitation of photosynthesis in nitrogen deficient plants appears to be the result of complex regulatory mechanisms. The primary limitation seems to be due to a decrease in the content of RuBPCO which comprises the main proportion of soluble protein. However, the activities of enzymes which catalyze the formation of RuBP from TP appear to be decreased as well. This results in a decreased RuBP level and in increased assimilatory power. RuBPCO is activated. It is suggested that RuBPCO activity is more or less directly affected by the amount of assimilatory power. RuBP carboxylation appears to be the limiting step of photosynthesis in nitrogen deficient plants. The increased degree of RuBPCO activation appears to enable maximum photosynthesis at the decreased enzyme protein contents.

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