Leaflet photosynthesis rate and carbon metabolite accumulation patterns in nitrogen-limited, vegetative soybean plants

J. Michael Robinson
USDA, Agricultural Research Service, Beltsville Agricultural Research Center, Natural Resources Institute, Climate Stress Laboratory, Building 046A, Beltsville, MD 20705-2350, USA

Received 30 May 1996; accepted in revised form 23 September 1996

Key words: anaplerotic carbon metabolites, dark respiration, hexose phosphates, nitrogen-limitation, orthophosphate, photosynthesis, starch, sucrose

Abstract

Prolonged inorganic nitrogen (NO$_3^-$ + NH$_4^+$) limitation of non-N$_2$-fixing soybean plants affected leaflet photosynthesis rates, photosynthate accumulation rates and levels, and anaplerotic carbon metabolite levels. Leaflets of nitrogen-limited (N-Lim), 27-31-day-old plants displayed $\approx$15 to 23% lower photosynthesis rates than leaflets of nitrogen-sufficient (N-Suff) plants. In contrast, N-Lim plant leaflets displayed higher sucrose and starch levels and rates of accumulation, as well as higher levels of carbon metabolites associated with sucrose and starch synthesis, e.g., glycerate-3-phosphate and glucose phosphates, than N-Suff plant leaflets. Concurrently, levels of soluble protein, chlorophyll, and anaplerotic metabolites, e.g., malate and phosphoenolpyruvate, were lower in leaflets of N-Lim plants than N-Suff plants, suggesting that the enzymes of the anaplerotic carbon metabolite pathway were lower in activity in N-Lim plant leaflets. Malate net accumulation rates in the earliest part of the illumination period were lower in N-Lim than in N-Suff plant leaflets; however, by the midday period, malate accumulation rate in N-Lim plant leaflets exceeded that in leaflets of N-Suff plants. Further, soluble protein accumulation rates in leaflets of N-Suff and N-Lim plants were similar, and the rate of dark respiration, measured in the early part of the dark period, was higher in N-Lim plant leaflets than in N-Suff plant leaflets. It was concluded that during prolonged N-limitation, foliar metabolite conditions favored the channelling of a large proportion of the carbon assimilate into sucrose and starch, while assimilate flow through the anaplerotic pathway was diminished. However, in some daytime periods, there was a normal level of carbon assimilate channelled through the anaplerotic pathway for ultimate use in amino acid and protein synthesis.

Abbreviations: ADPG-PPiase-ADPglucose pyrophosphorylase; Ce-CO$_2$ in the leaf photosynthesis measuring cuvette; Cl-leaf internal CO$_2$ during photosynthesis measurement; Chl—chlorophyll; DHAP—dihydroxyacetone phosphate; GAP—glyceraldehyde-3-phosphate; Gsw—stomatal conductance with units as mmol H$_2$O m$^{-2}$ s$^{-1}$; G1P—glucose-1-phosphate; G6P—glucose-6-phosphate; F6P—fructose-6-phosphate; FBP—fructose-1,6-bisphosphate; FBPase-pH 8.1—chloroplastic fructose-1,6-bisP (C-1) phosphatase (pH 8.1); MAL—malate; N—inorganic nitrogen, i.e. NO$_3^-$ + NH$_4^+$ (at levels and molar ratios indicated); PE—post-emergence; PEP—phosphoenolpyruvate; PEPCase—phosphoenolpyruvate carboxylase; PGA—3-phosphoglycerate; PYR—pyruvate; PYR kinase—pyruvate kinase; Pn—net CO$_2$ photoassimilation in leaves; PPFD—photosynthetic photon flux density; PPRC—pentose phosphate reductive cycle; RuBP—ribulose-1,5-bisphosphate; rubisco—ribulose-1,5-bisphosphate carboxylase/oxygenase; SLW—specific leaf mass; SPS—sucrose-6-phosphate synthase; TCA cycle—tricarboxylic acid cycle; triose-P—DHAP + GAP

Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the United States Department of Agriculture, and does not imply its approval to the exclusion of other products or vendors that may also be suitable.
**Introduction**

In all green plants, a source of carbon skeletons for amino acid syntheses are organic acids withdrawn from the TCA cycle, e.g. oxalacetate and α-ketoglutarate; these acids are employed to support reductive amination and transamination to form amino acids which, in turn, support protein syntheses in leaves (Bassham et al. 1981; Champigny and Foyer 1992; Huppe and Turpin 1994; Touraine et al. 1992; Imsande and Touraine 1994). During elevated demands by green plant N assimilatory processes for carbon skeletons, e.g. when inorganic nitrogen ions are absent, there is rapid withdrawal of organic acids from the TCA cycle to support amino acid syntheses. Replenishment of acids in the TCA cycle is supported by anaplerotic anabolism from triose phosphates and PGA to PEP and PYR, ultimately resulting in the formation of OAA and malate (Paul et al. 1978; Bassham et al. 1981; Melzer and O’Leary 1987; Champigny and Foyer 1992; Mahn et al. 1993; Huppe and Turpin 1994). When N assimilatory demands are low, e.g. when NO$_3^-$ and/or NH$_4^+$ is present at limited levels, then anaplerotic enzyme activities are diminished to the extent that carbon assimilation shifts to favor elevated synthesis and accumulation of sucrose and starch (Robinson and Baysdorfer 1985; Rufty et al. 1988; Champigny and Foyer 1992; Huppe and Turpin 1994). When N assimilatory demands are low, e.g. when NO$_3^-$ and/or NH$_4^+$ is present at limited levels, then anaplerotic enzyme activities are diminished to the extent that carbon assimilation shifts to favor elevated synthesis and accumulation of sucrose and starch (Robinson and Baysdorfer 1985; Rufty et al. 1988; Champigny and Foyer 1992; Huppe and Turpin 1994). When N assimilatory demands are low, e.g. when NO$_3^-$ and/or NH$_4^+$ is present at limited levels, then anaplerotic enzyme activities are diminished to the extent that carbon assimilation shifts to favor elevated synthesis and accumulation of sucrose and starch (Robinson and Baysdorfer 1985; Rufty et al. 1988; Champigny and Foyer 1992; Huppe and Turpin 1994). When N assimilatory demands are low, e.g. when NO$_3^-$ and/or NH$_4^+$ is present at limited levels, then anaplerotic enzyme activities are diminished to the extent that carbon assimilation shifts to favor elevated synthesis and accumulation of sucrose and starch (Robinson and Baysdorfer 1985; Rufty et al. 1988; Champigny and Foyer 1992; Huppe and Turpin 1994).

Transfer of excised leaves, isolated leaf cells, or algal cells from N-sufficient nutrient solutions to nutrient solutions devoid of NO$_3^-$ and/or NH$_4^+$ results in a decrease in anaplerotic carbon metabolism and an increase in sucrose synthesis in the leaves or cells (Champigny and Foyer 1992; Huppe and Turpin 1994; Huber et al. 1994; Imsande and Touraine 1994). This has been shown to be caused by metabolite regulation and/or post-translational regulation of the activities of anaplerotic enzymes such as PEPcase and by regulation of the activities of sucrose synthesizing enzymes such as SPS (Champigny and Foyer 1992; Mahn et al. 1993; Huber et al. 1994; Huppe and Turpin 1994; Duff and Chollet 1995). For example, when excised leaves of N-sufficient wheat plants were shifted to incubation solutions where NO$_3^-$ was absent, activities of PEPcase decreased and activities of SPS increased due to the rapid dephosphorylations of these two enzymes (Champigny and Foyer 1992; Mahn et al. 1993; Huber et al. 1994; Duff and Chollet 1995). The accompanying physiological response was an increase in sucrose synthesis as well as a decrease in anaplerotic anabolism, e.g. decreased organic acid and amino acid synthesis (Champigny and Foyer 1992; Mahn et al. 1993).

Short term N-limitation of plants initially grown with sufficient nitrogen, or short term N-limitation of plant organs and cells derived from N-sufficient plants, has served as an excellent tool to examine mechanisms by which N assimilation influences carbon metabolism (Bassham et al. 1981; Champigny and Foyer 1992; Huppe and Turpin 1994; Duff and Chollet 1995). But the question remains whether these short term responses of carbon assimilatory processes, brought on by the rapid transfer of cut leaves or leaf cell isolates to incubation mixtures where NO$_3^-$ and/or NH$_4^+$ are absent, are similar to the responses of carbon metabolism induced by long term N stress of whole plants. Under field conditions, soil N level may limit plant growth, but soil is seldom, if ever, totally devoid of inorganic N; there is always some N available in the soil to support even minimal plant growth, especially during and just subsequent to irrigation or rain (Harper 1987).

Whole plant studies have been done which have examined the influence of prolonged N-limitation on sucrose and starch levels. For example, leaves of many plant species which have been adapted to growth limiting N levels for prolonged periods (e.g. ~30 or more days) display much higher daily levels of sucrose and lower soluble protein than leaves of N-sufficient plants (Robinson and Baysdorfer 1985; Oparka et al. 1987; Khamis and Lamaze 1990; de Veau et al. 1990, 1992; Rufty et al. 1984, 1988). Also, based on single time point determinations, leaves of N-limited, non-N$_2$-fixing soybean plants assimilate carbon metabolites in the anaplerotic pathway, although at reduced levels; this was reflected by the lower PYR kinase and PEPCase activities in leaflets of N-limited compared with those of N-sufficient plants (Robinson and Baysdorfer 1985). However it was not clear whether anaplerotic carbon flow was in any way limiting to the delivery of carbon skeletons to the TCA cycle, although amino acid levels were lower in the leaves of N-limited soybean plants (Robinson and Baysdorfer 1985).

The objective of the study was to more thoroughly elucidate how prolonged N-limitation of non-N$_2$-fixing, vegetative soybean plants affected both leaflet photosynthetic rate and the balance between the levels of foliar starch, sucrose, Pi, hexose phosphates and the levels of the anaplerotic metabolites, e.g. triose phosphates, PGA, PEP, PYR, and malate. The results indicated that in the leaflets of soybean plants subjected to several weeks of moderate N-limitation, there