Markers and signals associated with nitrogen assimilation in higher plants

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Abstract

A key concept underpinning current understanding of the carbon/nitrogen (C/N) interaction in plants is that the capacity for N assimilation is aligned to nutrient availability and requirements by the integrated perception of signals from hormones, nitrate, sugars, organic acids, and amino acids. Studies on the nature and integration of these signals over the last ten years has revealed a complex network of controls brokered by an interplay of C and N signals. These controls not only act to orchestrate the relative rates of C and N assimilation and carbohydrate and amino acid production, but they also have a significant influence on plant development. Amino acids are the hub around which the processes of N assimilation, associated C metabolism, photorespiration, export of organic N from the leaf, and the synthesis of nitrogenous end-products revolve. Since specific major amino acids or their relative ratios are modulated differentially by photorespiration and N assimilation, even though these processes are tightly intermeshed, they are potentially powerful markers for metabolite profiling and metabolomics approaches to the study of plant biology. Moreover, while minor amino acids show marked diurnal rhythms, their contents fluctuate in a co-ordinated manner. It is probable that factors associated with early events and processes in C and N assimilation influence the relative composition of minor amino acids.

Key words: Amino acids, carbon/nitrogen interaction, metabolite profiling, photorespiration, photosynthesis.

Introduction

The photoautotrophic production of organic nitrogenous compounds is crucial to plant metabolism, growth and development, and protein and amino acid contents of harvested parts are of great agronomic importance in many crop species. Light-driven N assimilation in leaves has evolved to operate alongside and intermesh with photosynthesis and respiration. The production of reduced C in photosynthesis and its reoxidation in respiration are necessary to produce both the energy and C skeletons required for the incorporation of inorganic N into amino acids. Conversely, N assimilation is required to sustain the output of organic C and N. This network is further complicated by the concomitant operation of photorespiratory metabolism. Over the last 30 years the numerous interactions between C and N metabolism have been intensively explored in organelles, tissues, whole plants, and even within populations, as the effects of global CO₂ enrichment became a topical issue. Such studies have indicated multiple levels of complex control and communication between different intracellular compartments, and revealed that changes in C and N status influence organ physiology and root/shoot relationships. It is becoming clear that both the rate of N assimilation and the co-ordination of C and N assimilation are under multifactorial control by a repertoire of signals which provide information on C and N status. This information is transduced into the appropriate regulation of gene expression and enzyme activities (Stitt et al., 2002; Foyer and Noctor, 2002). In this article, the identity and influence of such signals and the components that transduce them are reviewed. The use of metabolites

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Abbreviations: ABA, abscisic acid; Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartate; Fd, ferredoxin; GDH, glutamate dehydrogenase; Glg, glutamine; Glu, glutamate; Gly, glycine; GOGAT, glutamate synthase; GS, glutamine synthetase; GS₂, chloroplastic glutamine synthetase; ICDH, isocitrate dehydrogenase; Lys, lysine; NR, nitrate reductase; 2-OG, 2-oxoglutarate; PEPc, phosphoenolpyruvate carboxylase; PK, pyruvate kinase; Ser, serine.
involved in N assimilation as markers to characterize particular physiological states or processes is then discussed.

**Metabolic checkpoints in C/N co-ordination**

Two of the major metabolic checkpoints co-ordinating primary N and C assimilation in leaves are nitrate reductase (NR), controlling flux into N assimilation, and phosphoenolpyruvate carboxylase (PEPc), regulating organic acid production. These, and many other more minor checkpoints, are co-ordinated by metabolite cross-talk, substrate availability and provision of end-products. Since plants also reallocate C and N in response to developmental and environmental cues, other signals mediated by phytohormones, phytochrome and Ca\(^{2+}\) are also involved.

Nitrate reductase activity is an important control point. Maximal extractable NR activity is governed by multiple factors, the most important of which are light, nitrate, Gln, and sugars (Campbell, 1999; Stitt et al., 2002). The expression of *nia* genes, encoding NR, is induced by nitrate and sugars, and suppressed by exogenous Gln (Hoff et al., 1994). Transcript abundance is also under light/dark control, but is often out of phase with protein abundance. Moreover, *in vitro* NR activity does not correlate tightly with *nia* transcript abundance (Vincentz and Caboche, 1991). In addition, NR is subject to post-translational regulation through protein phosphorylation (Kaiser and Huber, 1994), and reversible binding of a 14-3-3 protein inhibitor (Bachmann et al., 1996). Light-activation involves de-phosphorylation followed by dissociation of enzyme and inhibitor protein, though the intermediates that link light/dark to changes in NR phosphorylation status remain obscure. While nitrate does not affect the phosphorylation status of NR, it does prevent decreases in NR capacity due to protein turnover (Ferrario et al., 1995, 1996). Compensatory modifications at several levels (including transcription, protein turnover, and phosphorylation status) dampened the impact of fewer *nia* genes on nitrate assimilation in tobacco (Scheible et al., 1997c). Several of the controls over NR activity seem to be affected by carbohydrate supply. As well as increasing *nia* transcripts, exogenous sugars influence the post-translational regulation of NR (Kaiser and Huber, 1994). Feeding sugars to tobacco leaves markedly stimulated nitrate reduction, an effect correlated both with greater stability of the NR protein and with an increase in NR activation state (Morcuende et al., 1998). Both increased stability and increases in activation state may be linked to sugar-induced decreases in NR phosphorylation status. Further work is required to identify the C metabolite(s) most important in controlling NR activity at these levels.

Phosphoenolpyruvate carboxylase catalyses the carboxylation of PEP to form oxaloacetate (OAA). It is a ubiquitous cytosolic enzyme with multiple roles in plants, including the photosynthetic fixation of CO\(_2\) in C\(_4\) and CAM plants. In the anapleurotic pathway, PEPc enables net C skeleton synthesis through the TCA cycle during inorganic N assimilation. The enzyme is activated by light and enhanced N supply (Champigny and Foyer, 1992; Foyer et al., 1994a). As for NR, light/dark modulation of PEPc activity is achieved by reversible protein phosphorylation. This modification, catalysed by PEPc protein kinase, changes the enzyme’s sensitivity to effectors, notably decreasing sensitivity to inhibition by L-malate (Vidal et al., 2002). The abundance of PEPc kinase mRNA is determined by metabolic triggers, particularly nitrate and compounds produced during C and N metabolism. Moreover, nitrate increases PEPc activity and favours the flow of C into amino acid synthesis with a concomitant reduction of sucrose synthesis via regulation of sucrose phosphate synthase activity (Van Quy et al., 1991; Sugiharto and Sugiyama, 1992; Foyer et al., 1994a). Changes in the activity of PEPc kinase appear to underpin the enhanced C flux to organic acids (Champigny and Foyer, 1992; Duff and Chollet, 1995).

**Integration of primary nitrogen and carbon metabolism**

Amino acid biosynthesis requires appropriate allocation of assimilated carbon, notably 2-oxoglutarate (2-OG) for ammonia incorporation, but also the production of C skeletons for amino acid synthesis downstream of the glutamine synthetase/glutamate synthase (GS/GOGAT) pathway. The proportion of fixed C required by N assimilation will change markedly according to developmental stage, N availability and the nature of the products (Lewis et al., 2000). Even low rates of N assimilation require diversion of a substantial part of fixed carbon to amino acid synthesis, both in source and sink leaves (Foyer and Noctor, 2002), and leaf starch accumulates when N is in short supply (Rufty et al., 1988). In tobacco, ADP-glucose pyrophosphorylase transcript abundance appears to be regulated by nitrate in an inverse fashion to the expression of enzymes involved in organic acid synthesis (Scheible et al., 1997b).

At the very least, net production of 2-OG involves part of glycolysis, PEPc, and three enzymes of the TCA cycle, or other isoforms of aconitase and isocitrate dehydrogenase (ICDH). High nitrate promotes organic acid synthesis, accompanied by enhanced expression of C\(_3\)-type PEPc (Scheible et al., 1997b, 2000). Changes in PEPc activity produce a significant shift from dissipatory respiration in the dark to anapleurotic respiratory flow in the light. It might be expected that PEPc activity would be regulated by C/N metabolites in an inverse fashion to NR (i.e. induced by Gln and other amino acids, repressed by organic acids). Indeed, the observed strong positive correlation between leaf Gln and PEPc activity (Murchie
et al., 2000) suggests an intimate relationship between N assimilation and increased flow through the anaplerotic pathway in both C₃ and C₄ plants.

From the perspective of leaf C/N balance, it can be predicted that a shortfall in C skeletons should be signalled back to nitrate reduction, reining in N assimilation and avoiding excessive production of nitrite and/or ammonia. Insufficient anaplerotic C in the light, where N assimilation is relatively fast, could result from (1) incapacity of the system to divert enough sugar-P to oxidation, for example, inadequate activity of enzymes such as pyruvate kinase (PK), pyruvate dehydrogenase and PEPc and (2) further oxidation of key acceptors such as 2-OG. Do appropriate signals ensure that high rates of nitrate reduction are coordinated with the anaplerotic production of C skeletons? Relevant data have been provided by a study of tobacco plants during the day/night cycle (Scheible et al., 2000). In mutants and transformants with low NR activity which accumulate high nitrate, changes in NR transcripts correlated with PEPc transcripts, though less well with those for PK, citrate synthase, and cytosolic ICDH (Scheible et al., 2000). In wild-type tobacco on high nitrate, however, a good antiparallel correlation was observed in the light period between the extractable activities of NR and and those of PK and ICDH (Scheible et al., 2000). By contrast, light-dependent changes in PEPc activity, though small, were in phase with NR activity. On the basis of these data and metabolite analysis, the authors suggested that, during the first part of the light period, PEPc activity acts mainly to generate malate as a counterion to balance pH during high rates of nitrate assimilation, whereas the production of C skeletons for the incorporation of assimilated ammonia occurs later in the light period (Scheible et al., 2000). Temporal offset of anapleurosis and N assimilation could facilitate allocation of C to amino acid synthesis, particularly at high rates of nitrate assimilation. In younger, sink leaves the system could be constructed to allow significantly higher allocation of C to amino acid synthesis during high rates of N assimilation.

Control over NR has to be distinguished from control over nitrate reduction. Constitutive expression of NR in tobacco does not affect chlorophyll contents, protein levels, photosynthesis or biomass, although increased Gln contents point to some effect on flux (Foyer et al., 1994b; Ferrario et al., 1995). Redox coupling could influence the integration of N assimilation and C metabolism. Even in the simplest case, where anaplerotic C oxidation occurs from triose-phosphate and the oxidative pentose phosphate pathway is not involved (as could perhaps mainly be the case in illuminated leaves), the net formation of one 2-OG would involve net production of four NAD(P)H, two to three of which could be formed in the cytosol, depending on the location of isocitrate oxidation. If nitrate acts as a cytosolic electron acceptor for one-quarter of the reductant formed, there is still an excess that must be oxidized by other means, for example, via the mitochondrial electron transport chain. Insufficient sinks for reductant could both stimulate nitrate reduction and hamper the production of 2-OG, causing accumulation of Gln and, possibly, NH₃, in the chloroplast (see below). Even when assay conditions measure only the active dephosphorylated form of NR, this activity is almost always in excess of in vivo nitrate reduction, suggesting limitation by either nitrate or NAD(P)H (Kaiser et al., 2002). Limitation of NR by cytosolic reductant could be one, often overlooked, factor that ensures that nitrate reduction does not proceed at rates that are too far in excess of the supply of 2-OG.

Metabolite signals in C/N interactions

The two most common approaches to unravelling the identity of signals in the C/N interaction have been the analysis of abundance of key transcripts, either in mutants/antisense transformants or after feeding of substrates or metabolites. While feeding experiments have not always been accompanied by comprehensive analysis of the effect of the compound supplied on the tissue concentrations of related metabolites, studies of mutants and transformants can lead to overestimation of the importance of a given signal in wild-type plants. Nevertheless, such studies have identified a number of important signal compounds, notably including Gln (Vincentz et al., 1993) and nitrate (Scheible et al., 1997a, b).

Nitrate induction of NR and the enzymes involved in organic acid synthesis is a feedforward activation of downstream pathways signalled by increased substrate availability. What are the factors that feed back on nitrate reduction and regulate organic acid synthesis? The short-term effects of these factors could be less influential than previously thought, if temporal decoupling of N assimilation and anaplerotic organic acid synthesis is a general phenomenon. There is conflicting evidence concerning the role of Gln in controlling nitrate reduction and organic acid synthesis. Although supplying Gln by the transpiration stream caused repression of nia transcripts in Arabidopsis, no repression was associated with the accumulation of Gln in Fd-GOGAT mutants (Dzuibany et al., 1998). These observations were reconciled by invoking an indirect effect of exogenous Gln on NR expression, mediated via decreased nitrate concentrations (Dzuibany et al., 1998). This hypothesis cannot, however, account for repression of NR transcripts in sulphur-limited tobacco, where Gln and Asn accumulated, but leaf nitrate remained relatively high (Migge et al., 2000). An alternative explanation is that the effectiveness of Gln as a signal is amplified by the plant cell’s possible capacity to sense 2-OG, so that the Gln/2-OG ratio is an important regulatory parameter, as in bacteria and fungi. Hence, the effects of increases in Gln in Fd-GOGAT mutants may be offset by the antagonistic
influence of increased 2-OG. Concomitant changes in Gln and 2-OG were observed in tobacco lines where a range of Fd-GOGAT activities was produced by antisense technology, both compounds increasing as Fd-GOGAT capacity was decreased (Ferrario-Méry et al., 2000). In agreement with the data of Dzuibany et al. (1998), the rise in Gln did not repress NR transcripts. In fact, NR transcripts increased as Fd-GOGAT capacity decreased (Ferrario-Méry et al., 2001). Feeding experiments showed that Gln decreased NR expression while sucrose had an opposing effect (Ferrario-Méry et al., 2001). A new observation was that supplying 2-OG, which caused a 2-fold increase in leaf 2-OG contents, had a similar effect on NR transcripts to supplying sucrose (Ferrario-Méry et al., 2001). These data suggest that antagonistic effects of Gln and 2-OG are able to explain the lack of NR suppression accompanying Gln accumulation in plants with low GOGAT activity (Dzuibany et al., 1998; Ferrario-Méry et al., 2001). Feeding experiments in the Stitt laboratory also produced some evidence for NR induction by 2-OG, although the effects were small and it was shown that the interpretation may be complicated by accompanying changes in other metabolites such as Gln and malate (Müller et al., 2001). In fact, an interesting observation was the repression of NR by feeding malate (Müller et al., 2001), which is consistent with the idea that accumulation of this organic acid is closely coupled to nitrate synthesis, primarily due to its role as a counterion.

In an extreme hypothetical case in which a given metabolite exerted rapid and effective control, the concentration of the metabolite would show little or no fluctuation. An important role for Glu has been suggested (Coruzzi and Zhou, 2001), consistent with the relative stability of overall leaf pools of this amino acid throughout the day/night cycle in tobacco (Stitt et al., 2002). The repression of NR transcripts was observed on supplying either Glu or Gln to detached tobacco leaves (Vincentz et al., 1993). In the absence of any characterization of the effects of feeding on leaf metabolite contents, such effects are difficult to interpret. Knock-on effects of Gln accumulation, such as increases in Asn, could also transmit information on the C/N balance (Migge et al., 2000). The permitted elasticity of any one component (e.g. the Gln pool) is effectively the inverse of the system’s sensitivity to changes in its value: on this basis, the literature data taken as a whole indicate that Gln is a signal that, at best, provides a relatively weak and/or delayed control over flux in the initial reactions of N assimilation. It is unlikely that any one factor, be it nitrate or Gln, exerts more than fractional control. Each factor that is sensed will work in the context of changes induced by several others.

The analysis of plants deficient in Fd-GOGAT revealed that the direction of the glutamate dehydrogenase (GDH) reaction varied during the day/night cycle, such that a higher ratio of aminating to deaminating activity occurred in the first half of the light period (Ferrario-Méry et al., 2002a). This was correlated with the decline in ammonia and 2-OG concentrations, consistent with an increase in amminating GDH activity in vivo. Such observations suggest that the ammonia assimilation pathway may be very flexible, and that pathways alternative to GS-GOGAT can be activated as required. A transfer to photorespiratory conditions also led to an activation of anapleurosis, as evident from increases in PEPc and ICDH protein amounts and activities (Ferrario-Méry et al., 2002b). By contrast, transcripts for PEPc were unaltered, as were those for both cytosolic and mitochondrial ICDHs (Ferrario-Méry et al., 2002b). PEPc activity correlated well with PEPc protein and with leaf Gln, suggesting that Gln may affect translation or protein stability (Ferrario-Méry et al., 2002b). It is interesting that PEPc protein should be induced even when marked accumulation of 2-OG occurs, emphasizing the potential influence on this enzyme of increases in Gln.

Signal transduction components

Four distinct N and/or C- sensing systems have been identified in higher plants to date. These monitor the accumulation of different molecules at the crucial checkpoints in metabolism described above. Glucose sensors such as hexokinase orchestrate carbohydrate metabolism in source and sink tissues, and balance supply and demand in carbohydrate-producing and consuming cells over a wide range of environmental conditions. Systems for sensing N include a PII-like element that acts as a C/N-sensor (Hsieh et al., 1998), putative glutamate receptors (aGLRs) that have similarities to animal ionotropic glutamate receptors (Lacombe et al., 2001), and ‘two-component regulatory systems’ or ‘multistep His-Asp phosphorelay’ systems. Amino acid-mediated regulation of gene transcription in plants may have similarities to the yeast system. Amino acid deficiency in yeast decreases protein synthesis and increases the expression of a number of amino acid biosynthetic genes. This process, involving at least 35 genes in the 12 different amino acid synthesis pathways, is known as ‘general amino acid control’ (Hinnebusch, 1994). The yeast pathway of amino acid sensing involves protein kinases. In particular, the GCN2 (General Control Non-reversible 2) factor is a kinase of major importance in amino acid signalling (Wek et al., 1989). GCN2-mediated phosphorylation of eIF-2 under conditions of amino acid deprivation increases the expression of amino acid biosynthesis genes through the action of a transcriptional activator, GCN4 (Hinnebusch, 1997). In turn, the amount of GCN4 protein appears to be regulated by translational controls (Hinnebusch, 1994).

While metabolite measurements provide some indication of a form of general control of amino acids in plants (Noctor et al., 2002), evidence in support of co-ordinated
regulation of genes encoding enzymes of amino acid biosynthesis is scarce. Blocking histidine biosynthesis in Arabidopsis thaliana increased the expression of eight genes involved in the synthesis of the aromatic amino acids, histidine, L-lysine, and purines (Guye et al., 1995). Similarly, genes encoding tryptophan biosynthesis pathway enzymes in Arabidopsis thaliana have also been shown to be induced by amino acid deficiencies (Zhao et al., 1998).

The signal transduction network that co-ordinates information from carbohydrate metabolism and N assimilation is subject to phytohormone regulation. Abscisic acid (ABA) and two ABA-sensing proteins, ABI4 and ABI5, are now thought to be involved in the co-ordinate sensing of sucrose, glucose and nitrate (Finkelstein et al., 1998; Huijser et al., 2000; Signora et al., 2001). Whether these proteins are directly involved in the sugar signal transduction network or whether they are more indirectly involved in regulating the responsiveness of plant tissues to sugars is still a subject of debate (Rook et al., 2001; Rook and Bevan, 2003). Moreover, the integration of information arising from nitrate signalling at the whole plant level involves at least three plant hormones: ABA, auxin and cytokinin.

The use of amino acids as metabolite markers

The term marker is used here to denote metabolites or related parameters whose tissue value provides significant information on the rate of a process or on a metabolic state (e.g., C/N status). Metabolome analysis incorporates amino acid measurements as components of more global analyses of leaf metabolism (Roessner et al., 2000) capable of either enlightening or confusing. What useful information can be gleaned from measuring a more restricted subset of compounds? Which parameters, if any, can be exploited as reliable markers?

Most concepts of metabolic control in higher plants incorporate the logic of homeostasis. Nitrate activation of N metabolism and related C metabolism (Scheible et al., 1997b) adjusts the rate of nitrate utilization to nitrate supply, while high nitrate down-regulates the growth of roots relative to shoots (Scheible et al., 1997b; Zhang et al., 1999). Homeostatic regulation is particularly evident in the notion that the primary organic products of N assimilation feed back to regulate nitrate reduction and feed sideways to adjust the rate of anapleurosis. It could therefore be predicted that the best markers for the rate of a process would be those that have least influence in signalling, i.e. powerful internal signals would be poor markers while good markers would exert little control. This may hold true for photorespiration (see below), probably because this process is controlled principally or exclusively by the rate of RuBP oxygenation and most other enzymes are usually in excess (Wingler et al., 2000), i.e. useful markers exist because the pathway is relatively little influenced by metabolite signals. For N assimilation, however, this view is probably far too simplistic, because a repertoire of substrates and metabolites interact, possibly with similar positive or negative weight, to influence gene expression and enzyme activities at multiple levels. Furthermore, it does not take into account the possibility of temporal offset or amplification/threshold/dampening effects in signalling and response. It is therefore possible that a given metabolite or related parameter can both act as a regulatory signal and serve as a reasonably useful marker.

From a theoretical point of view, it is extremely difficult to predict how leaf amino acid contents will be affected by any one of the numerous processes involved in their synthesis and turnover. Extensive data are therefore required to characterize, in a range of conditions, the intrinsic relationships between individual amino acids and the key processes in which they are produced, in order to produce essential standards against which future analyses can be calibrated. An obvious primary theoretical consideration is relative flux rates into an amino acid pool. For this reason, it has been proposed that leaf Gln will be more readily influenced by photosynthesis than by N assimilation, since the former can be more than 10-fold faster (Stitt et al., 2002). This is likely to be true when Gln pools undergo readjustment during transient changes in respiratory flux. As discussed below, different rates of photosynthesis may have less impact at the steady state. Another case is 2-OG content, which tends to increase somewhat as the steady-state rate of photosynthesis increases (Novitskaya et al., 2002). This effect occurs even though, theoretically, no net formation or utilization of 2-OG occurs during photosynthesis. It is probably due to the rapid production of glyoxylate or, possibly, increases in anapleurosis at high rates of photosynthesis (Ferrario-Méry et al., 2002b). In the following sections, three cases where parameters related to leaf amino acids could be useful markers are considered

Markers for photorespiration

The effects of N assimilation and photorespiration on amino acids in leaves with C3 photosynthesis have been considered to be inextricable (Stitt et al., 2002). While slow accumulation of Gly and Ser throughout the day (Scheible et al., 2000) suggests that the two processes are tightly linked, a recent examination of the influence of photorespiration on leaf amino acids found that three parameters showed a good correlation with photosynthetic flux acids (Novitskaya et al., 2002). A positive correlation between Gly/Thr and photorespiration was established while both Asp and Ala levels correlated negatively with photorespiration. It is noteworthy that though leaf Gly contents (on a chlorophyll or fresh weight basis) tend to increase with photorespiration, the relationship was less evident than that found when Gly was
expressed as a proportion of leaf amino acids. Furthermore, it was much poorer than the good relationship observed for Gly/Ser (Novitskaya et al., 2002). It is therefore likely that the absolute amounts of Gly and Ser are influenced by N status as well as by photorespiration (Scheible et al., 2000). The ratio Gly/Ser, however, correlates well with the flux of C through the photosynthetic pathway, regardless of the absolute amounts of each amino acid (Novitskaya et al., 2002). Studies of wheat, barley, potato, and tobacco leaves suggest that Gly/Ser, together with the percentage of amino acids present as Ala and, particularly, Asp, can provide a reasonable estimate of the relative rate of photorespiration in many C3 crop species (Novitskaya et al., 2002; Foyer and Noctor, 2002). This holds true as long as a rapid sampling technique is used that freezes metabolism at the in vivo state within a few seconds. It is noteworthy that the Asp concentration falls markedly in the light in barley leaf phloem (Lohaus and Fischer, 2002). Hence, the rate of photorespiration in the mesophyll cells is likely to be a significant factor influencing the composition of amino acids exported from source leaves in C3 species (Madore and Grodzinski, 1984).

Markers for primary nitrogen assimilation

Leaf amino acid contents generally correlate with N supply (Khamis et al., 1990; Scheible et al., 1997a). Similarly, in the short-term, amino acid contents increase when nitrate or ammonia are fed to excised leaves (Foyer et al., 1994a). Short-term effects probably mainly reflect increased substrate supply and/or enzyme activation while longer-term changes are also due to modified expression of enzymes such as NR (Scheible et al., 1997b). However, such short-term N-induced increases in amino acids are not general and are often dominated by the accumulation of one or two amino acids, particularly Gln (Foyer et al., 1994a). A striking correlation between extractable NR activity and Gln was found in tobacco mutants and transformants displaying a wide range of NR capacities, when grown on different nitrate concentrations (Scheible et al., 1997a). Other studies also support a strong influence of N assimilation on leaf Gln and/or Gln/Glu. Overexpression of NR in tobacco resulted in significantly increased Gln (Foyer et al., 1994b) while, in barley, Gln/Glu decreased in leaves suffering mild water deficits, even though photorespiration is probably increased under these conditions (Wingler et al., 1999). These data are consistent with the notion that, under some conditions at least, primary N assimilation affects Gln contents more than the rate of photorespiration does.

By manipulating conditions so as to change rates of photorespiration in concert with or independently of the rate of photosynthesis, it was shown that leaf Gln and Gln/Glu revealed no clear relationship with the calculated photorespiratory flux (Novitskaya et al., 2002). Both Gln and Gln/Glu were variable from leaf to leaf and correlated better with the rate of photosynthesis than with photorespiration. One explanation is that primary N assimilation is co-ordinated with photosynthesis and that the input of ammonium from this source affects leaf Gln more strongly than the faster but cyclic photorespiratory process. Key factors in determining leaf Gln (and Gln/Glu) are likely to be the rate of ammonia input and the relative rate of 2-OG supply, which in primary N assimilation are coupled relatively loosely compared to their tight coupling in photorespiration (Novitskaya et al., 2002). This view is consistent with other studies: when 2-OG was supplied to tobacco leaves, Gln contents fell, even though the extractable activity of NR was increased (Müller et al., 2001). Although control of ammonia assimilation by modulation of enzyme activities could also be important, it is not clear how modulation of the capacities of GS2 and Fd-GOGAT exerts appreciable control over N assimilation. In the leaves of C3 plants, the activities of these enzymes must be sufficient to cope with much higher rates of ammonia release during photorespiration. While not necessarily faithful indicators of the rate of N assimilation itself, Gln and Gln/Glu provide useful information on the balance between ammonia and 2-OG availability (Novitskaya et al., 2002; Foyer and Noctor, 2002). As for other amino acids involved in the photorespiratory cycle, accurate measurement of Gln/Glu in illuminated leaves requires rapid sampling. Gln can accumulate markedly during the first 30 s after darkening (G Noctor, CH Foyer, unpublished results), probably as a result of insufficient reduced Fd for GOGAT activity, or because ongoing ammonia release from Gly accumulated in the light exceeds the rate of 2-OG regeneration as glyoxylate availability falls, or both.

When tobacco with low Fd-GOGAT were transferred to air, there was accumulation of Gln, 2-OG and ammonia (Ferrario-Méry et al., 2002a). The nocturnal decrease in these compounds was accompanied by an increase in Asn, suggesting that this amino acid serves as a temporary storage compound for the elimination of excess photorespiratory ammonia (Ferrario-Méry et al., 2002a). In short-term experiments with wild-type wheat and potato, Asn did not correlate with either photorespiration or Gln contents (Novitskaya et al., 2002), although this does not rule out a build-up of Asn in longer-term experiments. Current knowledge suggests that a useful set of markers for N assimilation rates would include Gln, Gln/Glu, Gln/2-OG, together perhaps with malate/2-OG and, possibly, Asn.

Minor amino acids

Light stimulation of N assimilation produces a diurnal rhythm in total leaf amino acids. The exact nature of these
changes is likely to be species- and condition-specific, and it cannot be excluded that growth in constant environment chambers entrains or accentuates such fluctuations. Equally, such diurnal fluctuations could be less marked in younger leaves, where local sinks for amino acids are relatively powerful. In wheat leaves approaching the end of the sink/source transition, i.e. leaves with high rates of photosynthesis nearing full expansion, diurnal changes in total amino acids were accompanied by a concerted rhythm in minor amino acids (Foyer and Noctor, 2002).

Changes in minor amino acids were more marked than the overall modulation of total amino acids, so that the former increased significantly during the second half of the light period, both with respect to chlorophyll and as a fraction of total amino acids. Minor amino acid contents were lowest in the middle of the day and highest during the first part of the dark period. Extensive work in tobacco has also demonstrated marked diurnal changes in minor amino acids in this species (Stitt et al., 2002, and references therein). What causes these effects? Studies in tobacco have highlighted the potential significance of leaf carbohydrate metabolism in influencing amino acid contents. Minor amino acid contents were found to correlate with starch and sucrose, when carbohydrate accumulation was manipulated by day length (Matt et al., 2001). In an alternative approach, excised leaves were fed sucrose, which led to a general increase in several minor amino acids (Morcuende et al., 1998). The day/night changes in minor amino acids may be influenced by a build-up of carbohydrates and/or amides, or by the integrated transduction of several signals that serve as monitors of leaf C/N.

Minor amino acids are synthesized through distinct pathways that are under specific control by key enzymes (Morot-Gaudry et al., 2001). Contents are unlikely to be markedly affected by short-term changes in either photosynthetic C supply or photorespiratory rates, and this notion is confirmed by analysis of amino acids in wheat and potato leaves sampled under conditions of widely differing rates of photosynthesis and photorespiration. No correlation with photosynthetic parameters was observed, even though minor amino acids varied more than 20-fold between leaves under the different conditions (Noctor et al., 2002). The most striking aspect of the data was the good correlation between minor amino acids synthesized via different pathways (Noctor et al., 2002). Leaf contents of tyrosine, for example, correlated not only with leaf phenylalanine but also with leaf valine and leaf Arg (Noctor et al., 2002). Only part of this correlation could reflect the concerted diurnal rhythm in minor amino acids, since the experiments were carried out within a window of 4–6 h in the middle of the photoperiod, during which period the mean contents of minor amino acids varied about 2-fold. However, the same mechanisms that produce the diurnal rhythm in minor amino acids could also be responsible for the correlation observed in leaves incubated in different short-term conditions. Leaf carbohydrate contents are known to vary considerably, even between leaves sampled in identical conditions. Although processes other than synthesis may contribute to the changes in minor amino acids observed in tobacco, wheat and potato, it is an intriguing possibility that genes involved in minor amino acid synthesis might be controlled, at least in part, by carbohydrates or associated factors. Since phloem amino acid composition is thought to reflect that of the mesophyll cells (Lohaus and Fischer, 2002), one effect of co-ordinated contents will be to dictate delivery to sink tissues in equal relative amounts. In turn, the protein and amino acid contents of harvested organs such as seeds can be influenced by the composition of the phloem (Lohaus et al., 1998), highlighting the potential agronomic importance of understanding the factors that control leaf minor amino acid contents.

One explanation of the orchestrated variation in minor amino acids is that key enzymes are under common control by factors linked to primary N or C assimilation. Whatever the cause, the observed co-ordination of leaf amino acid contents, which the authors have observed in wheat, potato, barley, and tobacco, provides a useful standard against which to evaluate leaf C/N status, particularly in assessing the effects of mutations or transformations. Ultimately, such analyses might find an application in the selection of genotypes. Although the correlations observed between leaf minor amino acids hold for a given genotype on a given nutrient regime, recent observations suggest that factors which perturb the primary processes of C and N assimilation modulate these relationships, affecting the relative contents of individual minor amino acids differentially (authors’ unpublished results). It is intriguing to speculate that the initiation of such modulation involves parameters such as Gln/Glu or Gln/2-OG, and that these ratios could perhaps be more important in influencing downstream C/N metabolism than in controlling the initial processes of N assimilation.

References


