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MALATE METABOLISM AND ITS RELATION TO NITRATE ASSIMILATION IN PLANTS

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Key Word Index—Malate synthesis and oxidation; NADH oxidation; cytochrome and alternative oxidases; nitrate assimilation; adenylate control, light and dark reactions.

Abstract—The three enzymes phosphoenolpyruvate carboxylase, malate dehydrogenase and NAD malic enzyme-decarboxylating (ME) regulate the synthesis and oxidation of malate, which functions as a source of carbon for the anaplerotic operation of the citric acid cycle. Oxidation of malate is regulated by NADH, oxaloacetate and adenylate energy state of the cells. Coordinated reduction of nitrate by nitrate reductase (NR) and further assimilation of nitrite into amino acids is also controlled by these parameters and hence mitochondrial metabolism of malate regulates the nitrate assimilation pathway. The problems associated with the availability of NADH generated by mitochondria via ME for cytosolic NR are discussed. A unified concept for the regulation of light and dark assimilation of nitrate in the context of malate metabolism is presented.

INTRODUCTION

A close relationship between malate metabolism and nitrate assimilation in higher plants has long been recognised. Thus in whole plants Ben-Zeoni *et al.* [1] proposed that alkalization in leaves resulting from the assimilation of nitrate to produce ammonia and amino acids, stimulates the synthesis of malate, which is translocated into the roots via the phloem. Decarboxylation of malate generates HCO_3^- which is excreted into the medium, when NO_3^- is absorbed by the roots. This role of malate metabolism in the maintenance of ionic balance and pH has been reviewed by Davies [2] and Smith and Raven [3]. In this article, therefore, we wish to concentrate only on the recent advances in our understanding of the synthesis of malate in plant cells including the diverse pathways of its oxidation in mitochondria, in relation to the provision of reducing equivalents for the reduction and assimilation of nitrate. Recent work on the effects of the energy status of the cells on malate metabolism and its relation to the assimilation of nitrate in light and dark, both in leaves and roots will be briefly reviewed with a view to presenting a unified concept for the regulation of nitrate assimilation.

SYNTHESIS OF MALATE IN PLANTS

The central role of malate in plant metabolism has recently been reviewed by Lance and Rustin [4]. It has been emphasised that malate, a mobile storage form of CO_2 and reducing equivalents, can be oxidised by plant mitochondria without control by the cell energy charge. Malate, an intermediate in the citric acid cycle is synthesised by the action of fumarase, but a major source of malate in plant cells appears to be via what is now known as malate fermentation. In these reactions phosphoenolpyruvate (PEP), an intermediate in glycolysis is carboxylated by PEP carboxylase (PEPC) to produce oxaloacetate (OA), which in turn is reduced by NADH to malate in the presence of cytosolic malate dehydrogenase (MDH). The first major control point in glycolysis, namely phosphofructokinase is probably bypassed in photosynthetic cells on account of the export of triose phosphates (dihydroxy acetone phosphate; DHAP) or 3-phosphoglyceric acid (3-PGA) from chloroplasts via the phosphate translocator to the cytosol in counter exchange for inorganic phosphate. However, this control point cannot be bypassed in non-photosynthetic tissues such as roots. The second control point at the pyruvate kinase step can be bypassed in both roots and leaves by (phosphoenolpyruvate carboxylase, PEPC), to produce OA. NADH produced in this triose phosphate dehydrogenase step would then be recycled by MDH, producing malate. This pathway is self sufficient since both NAD^+ and ADP are recycled and there is no net change in these cofactors. The many faceted functions of PEPC in C_3 plants has recently been reviewed by Latzko and Kelly [5]. This enzyme is located in the cytosol of C_4 and CAM plants, but in C_3 plants, it is probably present both in the cytosol and chloroplast [6]. Although PEPC from CAM plants is reported to be inhibited by malate, the enzyme from C_3 plants is much less sensitive to malate. Hence large quantities of malate can be synthesised and subsequently stored in the vacuoles. Uptake of malate into vacuoles of mesophyll protoplasts of barley was shown to be energy dependent and stimulated by MgATP [7]. Influx of malate into vacuoles occurs in light only [7].

PATHWAYS OF MALATE OXIDATION

Malate stored in the vacuoles, as well as that being continuously synthesized in plant cells, both in light and dark, is considered to be a major source of carbon for the operation of the citric acid cycle in plant mitochondria. Palmer [8] has reviewed the recent work on the mechanism and regulation of malate oxidation in isolated plant mitochondria. An important aspect in which plant mitochondria differ from their animal counterparts, is the presence of a second enzyme of malate oxidation, namely the NAD-malic enzyme decarboxylating EC 1.1.1.39 (ME) in addition to MDH. The activity of ME allows the production of pyruvate from malate, thus facilitating the operation of the citric acid cycle, even under conditions when a glycolytic source of pyruvate is not available, due to the inhibition of glycolysis at one or both control points mentioned above. Both the malate oxidizing enzymes ME and MDH are now known to be located in the mitochondrial matrix space and the NADH generated in these reactions is oxidized via two pathways, namely a rotenone sensitive NADH dehydrogenase with a high affinity for NADH (K_m 8 μM) and a rotenone resistant NADH dehydrogenase, which has a low affinity for NADH (K_m 80 μM) which bypasses the first site of phosphorylation in the respiratory chain. Plant mitochondria also possess two pathways for electron transport to O_2, namely the cytochrome oxidase route, inhibited by antimycin A and cyanide, and the cyanide resistant alternative pathway, which is inhibited by salicyl hydroxamic acid (SHAM). Branching of the two pathways takes place at the level of ubiquinone, after complex I of the respiratory chain. Rustin *et al.* [9] suggested that NADH generated by ME could be oxidized via rotenone and cyanide resistant pathways by a completely non-phosphorylating mechanism. However, contributions of these different routes of malate oxidation in light and dark are not yet well understood. Recent work has demonstrated that concentrations of OA and NADH as well as the state of the adenylate charge, all control these reactions [10–12]. As pointed out by Neuberger *et al.* [11], an increase in the level of OA would result in the depletion of NADH concentration due to its oxidation by MDH. Under these conditions, only the rotenone sensitive NADH dehydrogenase, with a low K_m for NADH, would function. Thus the rate of rotenone resistant O_2 uptake during malate oxidation is a function of the matrix NADH levels, which in turn depend on the amounts of OA present. According to them [11], there is no need to postulate intra-mitochondrial compartmentation of these enzymes. When the cytochrome path is restricted by adenylates (state 4), as for example in light, the mitochondrial NADH level will rise, resulting in an inhibition of MDH and thus a low level of OA. Under these conditions, although pyruvate will be produced by ME, the citric acid cycle activity will be restricted because of the inhibition of OA supply. Although plant mitochondria have a specific carrier with high affinity for OA, the origin of this acid for entry into mitochondria is in doubt. Any OA produced by PEPC in the cytosol, would be immediately reduced to malate by NADH, which would be generated in the cytosol by the triose phosphate dehydrogenase step, which is a precursor of PEP. Perhaps direct export of 3-PGA rather than DHAP from the chloroplasts via the phosphate translocator would overcome this difficulty, because PEP can be produced from 3-PGA without the production of NADH. Under these conditions OA formed by carboxylation of PEP would not be reduced to malate so that it would be available for input into mitochondria. However, it has been reported [13] that the export of 3-PGA from the chloroplasts is restricted in light, probably because it is required in the Calvin cycle.

ANAPLEROTIC CARBON FLOW

In plant mitochondria, the citric acid cycle performs an anaplerotic function in order to supply carbon skeletons for amino acid synthesis and hence the problem of reoxidation of NADH generated in these reactions is very important. The non-phosphorylating electron transport via rotenone and cyanide resistant pathways would provide a mechanism for the turnover of the cycle in the presence of high levels of ATP. ME plays a crucial role in this anaplerotic function [14]. This enzyme is activated by CoA, which accumulates when pyruvate of glycolytic origin is not available for the synthesis of acetyl CoA. Day *et al.* [15] showed that CoA is taken up by the isolated plant mitochondria and, at higher pH, shifts malate metabolism from MDH to ME. ME activity is also regulated by the NAD/NADH ratio which, would permit mitochondrial respiration to continue by the anaplerotic functioning of ME when glycolysis has ceased [14]. Tobin and Givan [16], have shown that ATP inhibits MDH competitively with NAD^+ in mitochondria of mung bean hypocotyls and under these conditions OA levels are decreased. The cytosolic malate/aspartate ratio increases in light [17]. Due to the inhibition of MDH by ATP, it is likely that ME would become more involved in malate oxidation. However, as discussed above, an inadequate supply of OA would restrict the continuous activity of the citric acid cycle.

To summarize, the three enzymes, PEPC, MDH and ME, regulate the synthesis and oxidation of malate in plant cells. Oxidation of NADH generated by MDH and ME takes place via phosphorylating or non-phosphorylating pathways in the mitochondria. This process is regulated by the levels of NADH, OA and the energy charge of the system.

ASSIMILATION OF NITRATE

Nitrate reductase (NR, EC 1.6.6.1), the first enzyme in the assimilatory pathway is located in the cytoplasm of plant cells, although it may be loosely associated with the outer membrane of the chloroplasts [18]. Subsequent assimilation of nitrite is restricted to the plastids in roots and chloroplasts of leaves, where the enzymes nitrite reductase (NiR, EC 1.7.7.1), glutamine synthetase (GS, EC 6.3.1.2) and glutamate synthase (GOGAT, EC 2.6.1.5.3) are located [19, 20]. NADPH generated in the oxidative pentose phosphate pathway (OPPP) is probably the source of reductant for NR and GOGAT in the dark [21]. It has been shown that a controlled flow of carbon through OPPP, is dependent on the availability of sufficient glucose-6-phosphate (G-6-p) so that the induction of nitrite assimilation can occur [22]. In these reactions an essential role of ATP supply, which generates G-6-p via hexokinase has been established [23, 24]. Since free nitrite, which is toxic to plants, rarely accumulates in tissue under normal aerobic conditions, the rates of nitrate reduction and of subsequent assimilation of nitrite into amino acids must be strictly regulated. In non-

photosynthetic or darkened photosynthetic tissues, the control over nitrite assimilation is exercised by the supply of G-6-p, which in turn is dependent on ATP concentration. In darkness, the chloroplastic NiR as well as GOGAT utilize reduced ferredoxin (Fd) generated via ferredoxin-NADP reductase. In root plastids, although the presence of Fd-specific GOGAT has been reported [25], existence of chloroplast-type Fd has not been proven. It is likely that another non-haem iron protein with similar properties is involved for nitrite assimilation in plastids. This dark pathway of nitrite assimilation in chloroplasts is however switched off in light because OPPP is inhibited and is replaced by the Calvin cycle, reductive pentose phosphate pathway [26]. The key enzyme of OPPP, namely G-6-p dehydrogenase is known to be inhibited in light by thioredoxin [27–30].

Light dependent assimilation of nitrite in the chloroplasts is extremely rapid as compared with these reactions in the dark, because reduced Fd (for NR and GOGAT) and ATP (for GS) are directly generated by photosynthesis. Under both light and dark conditions, it is essential that cytosolic reduction of nitrate by NADH should be strictly regulated so that toxic nitrite does not accumulate in the tissues. Since nitrite assimilation is dependent on ATP supply in the dark and on photosynthetic electron transport in the light, reduction of nitrate must be initiated only under these conditions. As pointed out by Duke and Duke [31], the fact that NR activity is strictly controlled by light is not surprising because this regulation is absolutely essential for preventing damage to plants grown in light and supplied with nitrate. In roots, this control will be exercised by ATP supply (or ADP availability) via adenylate control of mitochondrial oxidation of NADH [32]. The possible mechanisms of these regulatory systems are discussed below.

SOURCES OF NADH FOR NITRATE REDUCTION

Whatever the precise location of NR in the cytoplasm, with or without an association with the chloroplast outer membrane, it is certain that NADH generated in the cytoplasm or in mitochondria is the source of reductant for the enzyme. The source of this reductant is however controversial [20], since it could arise in the cytosol via triose phosphates exported from chloroplasts [33], or from either chloroplast [34] or mitochondria [35] via dicarboxylate shuttles. However the capacity of these shuttles to export physiologically significant amounts of reducing equivalents from chloroplasts [36] or mitochondria [37] to the cytosol has been questioned.

On the basis of observed stimulation of nitrate reduction by citric acid cycle intermediates and inhibition by malonate, Sawhney *et al.* [38] suggested a mitochondrial origin of NADH for this reaction. Ramarao *et al.* [39] and Naik and Nicholas [40] also observed inhibition by malonate and D-malate and demonstrated a close relation between CO_2 evolution and nitrate reduction in leaves. Naik and Nicholas [40] found a 1:1 stoichiometry between CO_2 evolved and nitrite formed, and suggested that citric acid cycle dehydrogenases which generate CO_2 and NADH simultaneously are involved in providing reductant for nitrate reduction in leaves. An important role for mitochondrial ME was suggested. Whatever the source of NADH, abolition of competition for these reducing equivalents between mitochondrial respiration and NR seems to play a regulatory role in the assimilation of nitrate [41]. It has been established that massive reduction of nitrate to nitrite occurs when whole plants or plant tissues are placed under strictly anaerobic conditions since the reaction is highly sensitive to traces of O_2 [41]. However, even in air nitrate is readily reduced provided the mitochondrial electron transfer chain is inhibited at different sites by rotenone, amytal, antimycin A or CO [41, 42]. It was recently suggested that in roots of wheat, mitochondrial dehydrogenases in general and ME in particular also supply NADH for nitrate reduction [43]. This mechanism ensures a reduction of nitrate only when sufficient ATP is available to sustain an adequate supply of G-6-p to the plastids, for the assimilation of nitrite to glutamate.

It thus appears that both in roots and leaves, generation of NADH in the mitochondria and prevention or retardation of its oxidation by O_2 via the mitochondrial electron transfer chain are important factors which regulate nitrate reduction. However, the mitochondrial inner membrane is reported to be impermeable to the export of matrix generated NADH. Earlier suggestions of a mechanism of export of this NADH via a transmembrane transhydrogenase [44], and the location of ME within the intermembrane space [45] are no longer considered valid [46]. Although the import of externally supplied NADH, NAD^+, CoA and thiamin pyrophosphate into mitochondrial matrix is firmly established [4], direct export of internal NADH is not considered possible. Hence the problem of availability of mitochondrially-generated NADH for the reduction of nitrate in the cytosol remains unresolved. In light, the cytosolic reductant is known to increase and this is reflected by an increase in the malate/aspartate ratio [47]. Peine *et al.* [48] have monitored the pyridine nucleotide pattern in wheat leaves under different photosynthetic treatments. They observed a significant decrease in NADH concentration by about 60% after a 16 hr dark treatment and a subsequent increase in light. However the total NADH content of the leaves was less than 7 nmol/g, which is insufficient to account for *in vivo* nitrate reduction rates, which could be as high as 20 μmol/g/hr. Thus the absolute content of NADH in leaf tissues is of little importance and a continuous NADH generating system is required to account for the observed high NR activity. Cytosolic MDH could serve as a source of NADH, provided OA generated in the reaction as well as that produced by PEPC in the cytosol is rapidly removed, either by transamination or citrate synthase in the mitochondria in the presence of acetyl CoA generated by ME and pyruvate dehydrogenase.

However, since malate itself is synthesized in the cytosol via PEPC at the expense of cytosolic NADH, it is doubtful whether MDH can serve as a source of net NADH synthesis.

As shown earlier, ME plays a crucial role in the anaplerotic function of the citric acid cycle. Recently, Ruffner *et al.* [49] clearly demonstrated a precursor product relationship between photosynthetically derived 3-PGA and malate, via PEPC in grape leaf discs. However for the synthesis of malate, cytosolic NADH is consumed as follows:

$$1.\ 2\ PEP + 2\ CO_2 \xrightarrow{PEPC} 2\ OA.$$

$$2.\ 2\ OA + 2\ NADH + 2\ H^+ \xrightarrow{MDH} 2\ malate + 2\ NAD^+$$

After entering the mitochondrial matrix, malate is then oxidized as follows:

3. $\text{Malate} + \text{NAD}^+ \xrightarrow{\text{MDH}} \text{OA} + \text{NADH} + \text{H}^+$

4. $\text{Malate} + \text{NAD}^+ \xrightarrow{\text{ME}} \text{pyruvate} + \text{CO}_2 + \text{NADH} + \text{H}^+$

5. $\text{Pyruvate} + \text{NADH}^+ + \text{CoA} \xrightarrow[\text{dehydrogenase}]{\text{pyruvate}} \text{acetyl CoA} + \text{CO}_2 + \text{NADH} + \text{H}^+$

OA and acetyl CoA are used for citrate and isocitrate synthesis, and isocitrate is then oxidized to 2-oxoglutarate:

6. $\text{Isocitrate} + \text{NAD}^+ \xrightarrow[\text{dehydrogenase}]{\text{isocitrate}} \text{2-oxoglutarate} + \text{CO}_2 + \text{NADH} + \text{H}^+$

Thus while two moles of cytosolic NADH are consumed for malate synthesis (reaction 2), during subsequent oxidation of malate to generate 2-oxoglutarate, 4 NADH are produced (reactions 3, 4, 5 and 6). Thus there is a net gain of 2 NADH, which if exported to the cytosol could be available for NR. The 2-oxoglutarate can be used for glutamate synthesis in the chloroplasts. In non-photosynthetic tissues also a similar anaplerotic function of the citric acid cycle with malate generated via reactions 1 and 2 as the source of carbon has been postulated [50]. Day and Lambert [51] have shown that in roots, adenylate respiratory control of cytochrome oxidase can be tighter than that of glycolysis. Glycolytic flexibility can be achieved on by-passing the pyruvate kinase step via PEPC and MDH. Malate generated in these reactions can then be decarboxylated by ME in the mitochondria.

In photosynthetic tissues, DHAP exported from the chloroplasts is largely used for the synthesis of sucrose in the cytoplasm and it is not certain how much of it would be available for the synthesis of malate via 3-PGA and PEP. Moreover, the activity of cytosolic triose phosphate dehydrogenase itself would be restricted on account of a depletion of NAD^+ and ADP. Under these conditions it has been suggested that photorespiratory substrates such as glyoxylate could function as a source of carbon for the citric acid cycle [52–55]. Thus malate could be synthesized by the condensation of acetyl CoA with glyoxylate by malate synthetase and isocitrate could be formed from glyoxylate and succinate by the reversal of isocitrate lyase. Malate and isocitrate thus synthesized could be oxidized by the partial reactions of the citric acid cycle from malate to acetyl CoA and isocitrate to succinate respectively. Thus catalytic quantities of acetyl CoA and succinate would suffice to sustain a continuous oxidation of glyoxylate, which is generated in large quantities during photorespiration. In these reactions one mole of glyoxylate would give rise to two each of CO_2 and NADH. If this NADH is exported out of the mitochondria it would be available for NR. Malipero *et al.* [56] have also indicated that a precursor–product relationship exists between metabolites of photorespiration on the one hand and malate on the other.

OXIDATION OF EXTERNAL NADH IN MITOCHONDRIA

Another way in which plant mitochondria differ from their animal counterparts is the capacity to oxidize exogenous NADH. Since NR would obviously use cytosolic NADH, the competition between O_2 and NO_3^- would be at the level of exogenous NADH oxidation. Two membrane bound dehydrogenases are involved in these reactions, one in the outer membrane and the other in the outer surface of the inner membrane [57]. Oxidation of NADH via inner membrane NADH dehydrogenase bypasses the rotenone sensitive first site of phosphorylation but involves the cytochrome oxidase pathway, as does the succinate oxidase complex. There is little evidence to suggest that it is oxidized via the cyanide insensitive alternative oxidase [58]. Thus oxidation of exogenous NADH in the mitochondria is under respiratory control at the second and third sites of phosphorylation. This mechanism ensures that NADH would not be oxidized when sufficient ADP is not available. NADH would then be available for NR. Since nitrite reduction in the dark is initiated only when sufficient ATP is available for the generation of G-6-p, under conditions of respiratory control, a supply of mitochondrial NADH would ensure that nitrate is reduced to nitrite only when sufficient energy is available for its further reduction. Thus reduction of nitrate and nitrite could be co-ordinated by the state of the energy charge of the cell.

Oxidation of exogenous NADH by plant mitochondria has been reported to be dependent on Ca^{2+} ions [59, 60]. Møller *et al.* [61] found that chlortetracycline in the presence of Ca^{2+} ions inhibited this reaction. Involvement of calmodulin in the oxidation of exogenous NADH has been suggested [61], but recently Schwitzguebel *et al.* [62] found that calmodulin may not be directly involved in potato mitochondria. However, they have suggested that since Ca^{2+} is required in the reaction, calmodulin may indirectly affect Ca^{2+} concentration in the intermembrane space of mitochondria and hence the activity of NADH dehydrogenase located at the outer surface of the inner membrane.

Sawhney *et al.* [39] found that in wheat leaves, when mitochondrial NADH oxidation was inhibited by CO in air or by anaerobic conditions under argon, almost an equal amount of nitrate was reduced to nitrite. Under CO treatment in air the cytochrome oxidase pathway is inhibited, but the alternative cyanide (or CO) insensitive reaction would be functioning, while under argon both pathways would be inhibited. The results therefore indicate that alternative oxidase is not involved in the regulation of NADH supply to the cytosolic NR and it is essential to inhibit or restrict the cytochrome pathway to O_2.

REGULATION OF LIGHT AND DARK NITRATE REDUCTION BY MALATE METABOLISM

When the glycolytic source of pyruvate is restricted on account of adenylate respiratory control in the dark or inhibition of dark mitochondrial respiration in the light [26, 53], malate is clearly a source of carbon for the anaplerotic function of the citric acid cycle to generate 2-oxoglutarate for amino acid synthesis. Under both conditions the assimilation of nitrite is also facilitated by an adequate supply of ATP in the dark, or in light by a direct utilization of photosynthetically generated reducing power in the chloroplasts. It is interesting to note that these are the very conditions under which ME is stimulated to perform its anaplerotic function and NADH is made available for NR. The observation that inhibition of ME in leaves [39, 40] or in roots [43] by D-malate and malonate also restricts *in vivo* nitrate reduction and the reversal of this inhibition by fumarate, clearly indicates the important role of ME in the regulation of NR. The dark pathway of nitrate assimilation is extremely slow, dependent as it is on adenylate control of mitochondrial respiration and on a supply of G-6-p for nitrite reduction. This pathway is inhibited in light because the key enzyme of OPPP, G-6-p dehydrogenase, is switched off by reduced thioredoxin [27–30]. The light pathway dependent on photosynthesis is extremely rapid because of excess supply of NADH, abolition of competition for reducing equivalents between O_2 and NO_3^- and a very rapid assimilation of nitrite in the chloroplasts by direct utilization of reducing power and ATP generated in the light reaction.

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REFERENCES

1. Ben-Zioni, A., Vaadia, Y. and Lips, S. H. (1971) *Physiol. Plant.* **24**, 288.
2. Davies, D. D. (1979) *Ann. Rev. Plant Physiol.* **30**, 131.
3. Smith, F. A. and Raven, J. A. (1979) *Ann. Rev. Plant Physiol.* **30**, 289.
4. Lance, C. and Rustin, P. (1984) *Physiol. Veg.* **22**, 625.
5. Latzko, E. and Kelly, J. (1983) *Physiol. Veg.* **21**, 805.
6. Perrot-Rechenmann, C., Vidal, J., Brulfert, J., Burlet, A. and Gadal, P. (1982) *Planta* **155**, 24.
7. Martinoia, E., Flugge, U. I., Keirns, G., Heber, U. and Heldt, H. W. (1985) *Biochim. Biophys. Acta* **806**, 311.
8. Palmer, J. M. (1984) *Physiol. Veg.* **22**, 665.
9. Rustin, P., Moreau, F. and Lance, C. (1980) *Plant Physiol.* **66**, 457.
10. Wiskich, J. T. and Day, D. A. (1982) *Plant Physiol.* **70**, 959.
11. Neuburger, M., Day, D. A. and Douce, R. (1984) *Physiol. Veg.* **22**, 571.
12. Wiskich, J. T. and Dry, I. B. (1985) in *Encyclopedia of Plant Physiology*, New Series, Vol. 18, pp. 281–313. Springer, Berlin.
13. Heber, U. and Heldt, H. W. (1981) *Ann. Rev. Plant Physiol.* **32**, 138.
14. Artus, N. N. and Edwards, G. E. (1985) *FEBS Letters* **182**, 225.
15. Day, D. A., Neuberger, M. and Douce, R. (1984) *Arch. Biochem. Biophys.* **229**, 253.
16. Tobin, A. K. and Givan, C. V. (1984) *Plant Sci. Letters* **34**, 51.
17. Jordan, R. H. and Givan, C. V. (1979) *Plant Physiol.* **64**, 1043.
18. Beevers, L. and Hageman, R. H. (1980) in *The Biochemistry of Plants* (Miflin, B. J., ed.) Vol. 5, pp. 115–168. Academic Press, New York.
19. Lee, R. B. (1980) *Plant Cell Environ.* **3**, 65.
20. Abrol, Y. P., Sawhney, S. K. and Naik, M. S. (1983) *Plant Cell Environ.* **6**, 595.
21. Emes, M. J. and Fowler, M. W. (1979) *Planta* **145**, 287.
22. Emes, M. J. and Fowler, M. W. (1983) *Planta* **158**, 97.
23. Dry, I. B., Wallace, W. and Nicholas, D. J. D. (1983) *Planta* **152**, 234.
24. Bourne, W. F. and Miflin, B. J. (1970) *Biochem. Biophys. Res. Commun.* **40**, 1305.
25. Suzuki, A., Vidal, J. and Gadal, P. (1982) *Plant Physiol.* **70**, 827.
26. Graham, D. (1980) in *The Biochemistry of Plants* (Davies, D. D., ed.) Vol. 2, p. 526. Academic Press, New York.
27. Lendzian, K. and Zeigler, H. (1972) in *Photosynthesis* (Proc. International Cong. Photosynthesis Research) (Ferti, G., Avron, M. and Melandri, A., eds) Vol. 3, pp. 1831–1838. W. Junk, The Hague.
28. Buchanan, B. B. (1980) *Ann. Rev. Plant Physiol.* **31**, 341.
29. Ashton, A. R., Brennan, T. and Anderson, L. E. (1980) *Plant Physiol.* **66**, 605.
30. Schiebe, R. and Anderson, L. E. (1981) *Biochim. Biophys. Acta* **636**, 58.
31. Duke, S. H. and Duke, S. O. (1984) *Physiol. Plant.* **62**, 485.
32. Laties, G. G. (1982) *Ann. Rev. Plant Physiol.* **33**, 519.
33. Klepper, L., Flesher, D. and Hageman, R. H. (1971) *Plant Physiol.* **48**, 580.
34. House, C. M. and Anderson, J. W. (1980) *Phytochemistry* **19**, 1925.
35. Woo, K. C., Jokinen, M. and Canvin, D. T. (1980) *Aust. J. Plant Physiol.* **5**, 123.
36. Giersch, C. (1982) *Arch. Biochem. Biophys.* **219**, 379.
37. Schmitt, M. R. and Edwards, G. E. (1983) *Plant Physiol.* **72**, 728.
38. Sawhney, S. K., Naik, M. S. and Nicholas, D. J. D. (1978) *Biochem. Biophys. Res. Commun.* **81**, 1209.
39. Ramarao, C. S., Srinivasan and Naik, M. S. (1981) *New Phytol.* **87**, 517.
40. Naik, M. S. and Nicholas, D. J. D. (1981) *Aust. J. Plant Physiol.* **8**, 515.
41. Sawhney, S. K., Naik, M. S. and Nicholas, D. J. D. (1978) *Nature* **272**, 647.
42. Naik, M. S., Abrol, Y. P., Nair, T. V. R. and Ramarao, C. S. (1982) *Phytochemistry* **21**, 495.
43. Naik, M. S. and Nicholas, D. J. D. (1984) *Plant Sci. Letters* **35**, 91.
44. Day, D. A. and Wiskich, J. T. (1974) *Plant Physiol.* **54**, 360.
45. Coleman, J. O. D. and Palmer, J. M. (1972) *Eur. J. Biochem.* **26**, 499.
46. Day, D. A., Neuberger, M., Douce, R. and Wiskich, J. T. (1983) *Plant Physiol.* **73**, 1024.
47. Walker, K. A., Givan, C. V. and Keys, A. J. (1984) *Plant Physiol.* **75**, 60.
48. Peine, G., Hoffman, P., Seifert, G. and Schlling, G. (1985) *Biochem. Physiol. Pflanzen* **180**, 1.
49. Ruffner, H. P., Brem, S. and Rast, D. M. (1983) *Plant Physiol.* **73**, 582.
50. Journet, E. P. and Douce, R. (1983) *Plant Physiol.* **72**, 802.
51. Day, D. A. and Lambert, H. (1983) *Physiol. Plant.* **58**, 155.
52. Naik, M. S. and Singh, P. (1980) *FEBS. Letters* **111**, 277.
53. Singh, P. and Naik, M. S. (1984) *FEBS Letters* **165**, 145.
54. Singh, P. and Naik, M. S. (1983) *Plant Sci. Letters* **30**, 9.
55. Singh, P., Krumar, P. A., Abrol, Y. P. and Naik, M. S. (1986)

Physiol. Plant. (in press).
56. Malipiero, U., Ruffner, H. P. and Rast, D. M. (1981) *Z. Pflanzen Physiol.* **104**, 243.
57. Palmer, J. M. and Møller, I. M. (1982) *Trends Biochem. Sci.* **7**, 258.
58. Day, D. A., Arron, G. P. and Laties, G. G. (1980) in *The Biochemistry of Plants* (Davies, D. D., ed.) Vol. 2, p. 197. Academic Press, New York.
59. Moore, A. L. and Ackerman, K. E. O. (1982) *Biochem. Biophys. Res. Commun.* **109**, 513.
60. Møller, I. M., Johnston, S. P. and Palmer, J. M. (1981) *Biochem. J.* **194**, 487.
61. Møller, I. M. Palmer, J. M. and Johnston, S. P. (1983) *Biochim. Biophys. Acta* **725**, 289.
62. Schwitzguebel, J. P., Nguen, T. D. and Siegenthaler, P. A. (1985) *Physiol. Plant.* **63**, 187.