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Carbon and Energy Sources for Fatty Acid Biosynthesis in Non-photosynthetic Plastids of Higher Plants

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Non-photosynthetic plastids are the main sites of biosynthesis of a number of important plant products, including starch and oil. However, in contrast to chloroplasts, these plastids lack the ability to harvest light energy and to fix CO_2 via photosynthesis. Hence, they depend on continuous supply of energy (ATP), reducing power (NADH and NADPH) and carbon skeletons to sustain their biosynthetic activities. Studies conducted in the recent past have indicated these plastids to contain the full complement of glycolytic and oxidative pentose phosphate pathways, enabling them to metabolize carbon compounds to meet the above requirement. Furthermore, these plastids have also been shown to interact with extraplastidial compartments to maximize their rates of carbon metabolism. This interaction is achieved mainly through a large number of translocators present in the inner membrane of these plastids. These include triose phosphate translocator, hexose phosphate translocator, malate translocator, adenylate translocator and dicarboxylate translocator. Based on the nature of the substrates transported to plastids through translocators, a model has been proposed for the synthesis of fatty acids in leucoplasts of developing embryos of oil seed crops.

Key Words: Non-photosynthetic plastids, Fatty acid biosynthesis, Carbon source, Energy and reducing power, Metabolite transport

roduction

stids in non-photosynthetic tissues of higher nts are the main sites for the biosynthesis of rch (Tyson & apRees 1988), fatty acids (Dennis 9) and amino acids (Emes & Fowler 1979). wever, in contrast to chloroplasts, these stids lack the ability to harvest light energy l to fix CO_2 via photosynthesis (Emes & Tobin 3), and therefore, depend on continuous sup- of ATP, reducing power and carbon skeletons sustain their biosynthetic activities. The im- tant question, therefore, is how non-photo- thetic plastids obtain these precursors. One of possibilities could be that these organelles tabolize carbon compounds through glycolytic l/or oxidative pentose phosphate pathways es & Tobin 1993, Emes & Neuhaus 1997). stidial glycolytic capacity has been demonstrated in amyloplasts of developing wheat endosperm (Entwistle & apRees 1988), leucoplasts of cauliflower buds (Journet & Douce 1985) and developing endosperm of castor bean (Simcox et al. 1977). The leucoplasts from pea roots (Emes & Fowler 1983), barley roots (Oji et al. 1985), cauliflower buds (Journet & Douce 1985) and developing *Brassica* seeds (Gupta & Singh 1997) contain the enzymes of the oxidative pentose phosphate pathway, namely glu-6-P dehydrogenase and 6-phosphogluconate dehydrogenase. In contrast, the activities of one or more of the enzymes of glycolytic or oxidative pentose phosphate pathway have been shown to be either absent or very low in non-photosynthetic plastids (Emes & Tobin 1993). The enzymes not detected include phosphoglycero-mutase, and NAD-glyceraldehyde-3-P dehydrogenase in pea root leucoplasts (Borchert et al. 1993,

Trimming & Emes 1993) and Sycamore amyloplasts (Frehner et al. 1990). Similarly, glu-6-P dehydrogenase could not be detected in Sycamore amyloplasts (Frehner et al. 1990) and leucoplasts of castor bean endosperm (Simcox et al. 1977, Nishimura & Beevers 1979). The absence of these enzymes will completely prevent the efficient flow of carbon through the glycolytic or oxidative pentose phosphate pathways, necessitating the interaction of non-photosynthetic plastids with extraplastidial compartments to maximize their rates of carbon metabolism. The situation has further been complicated as the level of enzymes in non-photosynthetic plastids vary with plant species, type of tissue and the developmental stage at which the tissue is examined (Emes & Tobin 1993, Dennis et al. 1991, Miernyk & Dennis 1992, Thom et al. 1998). This has, therefore, created controversies on the data generated by different workers. Furthermore, the regulation of these pathways in non-photosynthetic plastids is not well understood (Emes & Neuhaus 1997). Moreover, the translocators present in inner membrane of these plastids and involved in exchange of metabolites with other cellular compartments have not yet been fully characterized (Emes & Neuhaus 1997). Thus compared to the information on metabolism in chloroplasts, the current understanding of metabolism in non-photosynthetic plastids is relatively poor. This has been partly due to the fact that, until recent past, the isolation of plastids from non-photosynthetic tissues has been difficult and partly because the dominant aspects of metabolism in chloroplasts tend to be more uniform in their fundamental properties, whereas in non-photosynthetic tissues the complement of metabolic pathways varies considerably within and between species (Emes & Tobin 1993). The present review summarizes the current understanding of metabolism in non-photosynthetic plastids in relation to fatty acid biosynthesis with the hope that this information will help us to understand the mechanism of biosynthesis of storage lipid (oil) in embryos of developing seeds of oil-bearing crops.

Scheme for Fatty Acid Biosynthesis in Plastids

The pathway for de novo fatty acid biosynthesis has been studied extensively and is essentially the same in all plant tissues (Stumpf 1980, 1984, 1987, Harwood 1996, Browse 1997, Heldt 1997, Ohlrogge & Jaworski 1997). Comprehensive reviews on fatty acid metabolism (Harwood 1996), regulation of fatty acid metabolism (Ohlrogge & Jaworski 1997) and molecular biology and biotechnological aspects of plant fatty acids (Browse & Somerville 1994, Kinney 1994, Murphy 1994, Ohlrogge & Browse 1995, Topfer et al. 1995) have already appeared. The purpose of including this section here is to know the requirements in terms of carbon source, energy and reducing power for fatty acid biosynthesis in non-photosynthetic plastids.

The process of fatty acid biosynthesis involves enzymes in a sequence of repeating reactions of condensation, ketoreduction, dehydration and enoyl reduction (figure 1), to increase the chain length of fatty acids until 16 carbon long, which may be elongated further to yield C-18 fatty acid, or may be used within plastid for glycerolipid biosynthesis or hydrolysed to free fatty acid by a soluble acyl-ACP thioesterase and exported from the plastids (figure 2). Acetyl CoA is the precursor for fatty acid biosynthesis, which arises as a result of oxidation of pyruvate by pyruvate dehydrogenase complex localized in the plastids (Emes & Neuhaus 1997). Fatty acid synthesis starts with the carboxylation of acetyl CoA by acetyl CoA carboxylase, with the consumption of ATP to yield malonyl CoA. In a subsequent reaction, CoA is exchanged through malonyl transacylase for acyl carrier protein (ACP). The enzyme β-keto-acyl ACP synthase III catalyzes the condensation of acetyl CoA with malonyl ACP to form β-keto acyl ACP. The liberation of CO_2 makes this reaction irreversible. The acetoacetate, thus formed, remains bound as a thioester to ACP and is reduced by NADPH to β-D-hydroxyacyl ACP. Following the release of water, the carbon-carbon double bond formed is reduced by NADPH to acyl-ACP. The product is a fatty acid which stands elongated by two

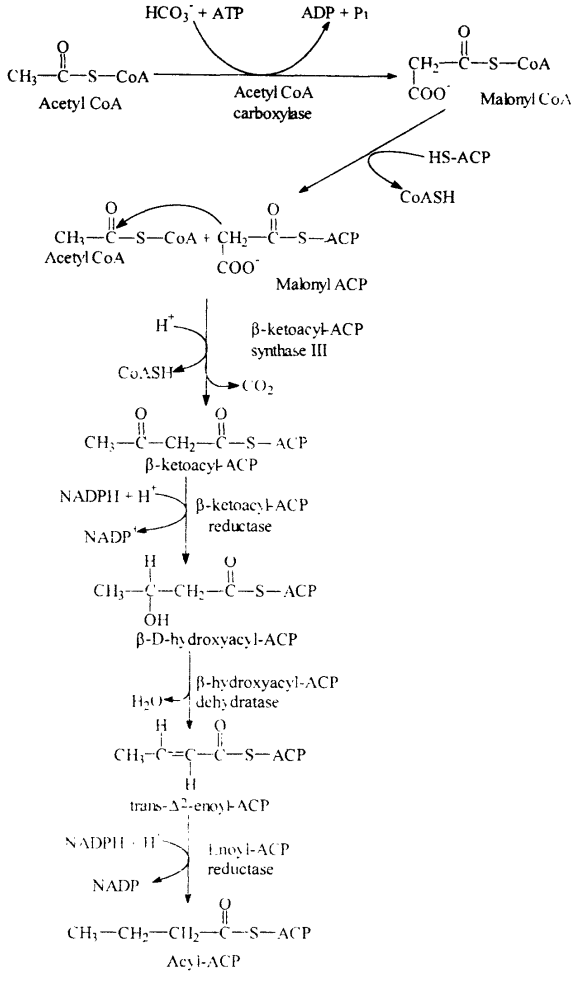

Figure 1 Reaction sequence for the synthesis of fatty acids: activation, condensation, reduction, release of water and further reduction elongate a fatty acid by two carbon atoms (Heldt 1997).

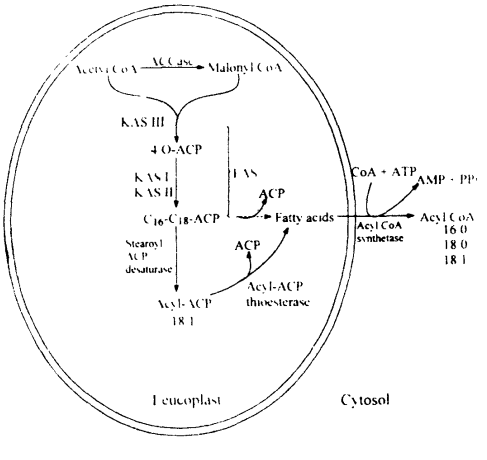

Figure 2 Simplified scheme for the overall flow of carbon through fatty acid in a generalized plant cell. Once the fatty acids leave plastid stroma and enter the cytosol, they are immediately converted to acyl CoA. *KAS*, β-ketoacyl-ACP synthase III; *FAS*, fatty acid synthetase; *ACP*, acyl carrier protein

carbon atoms. The chain is further elongated first by transferring it to another ACP and then condensing with malonyl-ACP. The enzyme β-keto acyl ACP synthase-I, catalyzing this reaction, enables the formation of fatty acids with a chain length of upto C-16. A further chain elongation to C-18 is catalyzed by β-keto acyl-ACP synthase-II. The stearoyl-ACP (18:0) formed in the plastid stroma is desaturated there to oleyl-ACP (18:1) by the enzyme stearoyl-ACP desaturase. The plastidial enzyme is capable of introducing only one double bond in fatty acids. The introduction of further double bonds is catalyzed by other desaturases, which are integral membrane proteins and react only with fatty acids which are constituents of membrane lipids. Acyl-ACP, before leaving the plastid, is hydrolyzed by acyl-ACP thioesterase to free fatty acids which leave the plastids. It is not yet clear whether this export proceeds via non-specific diffusion or by a specific transporter. The free fatty acids outside the plastid are immediately converted to acyl CoA by acyl CoA synthetases with consumption of ATP. Generally, the plastids mainly provide CoA-esters with the acyl residues of 18:1 and 16:0 (also a low amount of 18:0) for synthesis of various classes of lipids.

Acetyl-CoA carboxylase, the first enzyme of fatty acid synthesis, is an important regulatory enzyme and its reaction is regarded as a rate limiting step in fatty acid synthesis (Page et al. 1994, Ohlrogge & Jaworski 1997). In chloroplasts, the enzyme is active only during light and is inhibited during darkness (Browse et al. 1981). This explains why fatty acid synthesis in chloroplasts occurs only during the day when photosynthesis provides the necessary energy (ATP) and reducing nucleotides (NADPH). Light dependent changes in concentration of Mg^{2+} and pH in the stroma may also be involved in the regulation of this enzyme. The plastidial enzyme, consisting of multiple subunits, resembles the enzyme from cyanobacteria and other bacteria, and is referred to as the prokaryotic form of acetyl CoA carboxylase (Heldt 1997). The enzyme may also be present in cytosol where it may help

the chain elogation of fatty acids. However, in contrast to the prokaryotic form, the extra-plastidial enzyme consists of a single large multifunctional protein, and is referred to as the eukaryotic form.

The plant fatty acid synthetase is a type II dissociable complex from which the proteins catalyzing the partial reactions can be isolated and purified. The plastidial enzyme complex is similar to those of many bacteria and is called the prokaryotic fatty acid synthetase (FAS) complex. In contrast, the enzymes of fatty acid synthesis in animals and fungi are contained in only one or two multifunctional proteins, and termed as eukaryotic FAS complex. Further details of each component are beyond the scope of the present review.

Requirements for Fatty Acid Biosynthesis

The ability of non-photosynthetic plastids to synthesize fatty acids from labelled acetate has been studied in daffodil (Kleinig & Liedvogel 1978, 1980), maturing safflower and linseed cotyledons (Browse & Slack 1985), cauliflower buds (Journet & Douce 1985), developing castor bean seeds (Miernyk & Dennis 1983), germinating castor bean seeds (Vick & Beevers 1978), soybean suspension cells (Nothelfer et al. 1977), pea roots (Sparace et al. 1988, Stahl & Sparace 1991), cotyledons (Denyer & Smith 1988) and developing embryos of oilseed rape (Kang & Rawsthorne 1994) and *Brassica campestris* (Gupta & Singh 1996a). Fatty acid synthesis *in vitro* from (1-^{14}C) acetate in leucoplasts isolated from developing seeds of *Brassica campestris* was shown to be maximum when leucoplasts were incubated in a culture medium containing 0.8 mM acetate, 20 mM $NaHCO_3$, 8 mM ATP, 8 mM $MgCl_2$, 4 mM $MnCl_2$, 0.6 mM CoA, 1 mM NADH, 1 mM NADPH and 0.2 M sorbitol (Gupta & Singh 1996a). The rate of fatty acid synthesis in these preparations was highest at *p*H 8.5 in presence of 0.4 M Bistris-propane buffer and linear for 4 hr at 30°C with 80-110 μg plastid protein. ATP and divalent cations were absolute requirements, whereas nucleotides, CoA and bicarbonate improved the rate of fatty acid synthesis by two to ten folds. However, the degree to which the above cofactors are required for fatty acid synthesis varies with plastid types (Sparace & Kleppinger- Sparace 1993). In leucoplasts of rapidly growing root tissues of pea, fatty acid synthesis was maximum at 82 nmol hr^{-1} mg^{-1} protein in culture medium of 0.2M Na(1-^{14}C) acetate (11-14 mCi/mmol), 0.5 mM each of NADH, NADPH and CoA, 6 mM of ATP and $MgCl_2$, 1 mM each of $MnCl_2$ and glycerol-3-P, 15 mM $KHCO_3$, 0.31 M sucrose and 0.1 M Bistris propane (*p*H 8.0) incubated at 35°C (Stahl & Sparace 1991). In leucoplasts from castor endosperm, it required slight change in conditions that were required for fatty acid synthesis in pea root leucoplasts (Smith et al. 1992). Under *in vitro* conditions, at the standard incubation temperature of 25-30°C, the rate of fatty acid synthesis is linear upto 4-6 hr with 80-120 μg plastid protein (Stahl & Sparace 1991, Gupta & Singh 1996a). *p*H has a pronounced effect on the proportion of ^{14}C incorporated into fatty acids from (1-^{14}C) acetate (Nothelfer et al. 1977, Vick & Beevers 1978, Kleinig & Liedvogel 1980, Stahl & Sparace 1991). In pea root leucoplasts, *p*H affects the type of fatty acid synthesized and maximum rate of fatty acid synthesis was observed between *p*H 7.5 to 8.5 (Stahl & Sparace 1991). In these plastids, the rate limiting step in de novo fatty acid synthesis is the one catalyzed by stearate desaturase. This was further demonstrated by continuous decrease in total fatty acid biosynthesis with increasing concentrations of DTT upto 0.5 mM, as DTT is a known inhibitor of stearate desaturase (Stahl & Sparace 1991). ATP and divalent cations are the absolute requirements for fatty acid synthesis in non-photosynthetic plastids (Sparace & Kleppinger-Sparace 1993). ATP (3-4 mM) contributed to about two fold improvement in the rate of fatty acid synthesis in plastids from soybean suspension cells (Nothelfer et al. 1977) and daffodil chromoplasts (Kleinig & Liedvogel 1978), respectively. However, for such an improvement, as high as 8 mM ATP is required for leucoplasts from safflower

cotyledons (Browse & Slack 1985) and developing embryos of *Brassica campestris* (Gupta & Singh 1996b). In leucoplasts from pea roots, 6 mM ATP was shown to be optimum for fatty acid synthesis (Kleppinger-Sparace et al. 1992). In almost all preparations, low concentrations of Mn^{2+} and high concentrations of Mg^{2+} are needed for maximum rate of fatty acid synthesis. Higher amounts of fatty acids are synthesized with Mg^{2+} as compared to that with Mn^{2+}. ATP and Mg^{2+} in equimolar concentrations of 6 mM each gave higher activity in different preparations (Stahl & Sparace 1991, Kleppinger-Sparace et al. 1992, Gupta & Singh 1996a). Leucoplasts from pea roots (Stahl & Sparace 1991) and soybean suspension cells (Nothelfer et al. 1977) showed almost absolute dependence at CoA, whereas daffodil chromoplasts (Kleinig & Liedvogel 1980), and leucoplasts from cauliflower buds (Journet & Douce 1985) and developing castor bean seeds (Miernyk & Dennis 1983) were dependent to only 50, 15 and 10% extent on exogenous supply of CoA. In contrast, leucoplasts from developing embryos of *Brassica campestris* (Gupta & Singh 1996a) showed an essential requirement for CoA, giving only 10% of the activity in its absence. The greatest increase in activity is generally achieved with low concentrations of CoA, achieving 90% of the activity with 0.2 mM CoA.

Exogenous supply of reduced nucleotides, though is not an essential requirement for fatty acid biosynthesis in non-photosynthetic plastids, the rate increases when NADH and/or NADPH are supplied in the reaction mixture for fatty acid synthesis (Stahl & Sparace 1991, Qi et al. 1995, Gupta & Singh 1996a). In case of leucoplasts from developing seeds of *Brassica campestris* (Gupta & Singh 1996a), inclusion of both NADH and NADPH in equimolar concentrations (1 mM each) greatly stimulated fatty acid biosynthesis. However, NADH always gives higher activity than NADPH (Gupta & Singh 1996c), as is a general trend with all non-photosynthetic plastids (Sparace & Kleppinger-Sparace 1993). Addition of the two nucleotides together in equimolar concentrations always gave slightly higher activity than when they were used singly. Leucoplasts from sunflower cotyledons, however, had no apparent requirement for either of the nucleotides (Browse & Slack 1985). The above information on cofactor requirement for fatty acid biosynthesis in non-photosynthetic plastids is summarized in table 1. The variability observed here in the data could be explained in terms of the abilities of different plastids to retain and/or synthesize these cofactors during isolation as well as incubation.

Metabolic Sources of Energy and Reduced Nucleotides

The biosynthesis of fatty acids from acetate in all types of plastids requires the supply of energy (ATP) and reduced nucleotides (NADH & NADPH). While ATP is required for the synthesis of acetyl CoA and malonyl CoA by acetyl CoA synthetase and acetyl CoA carboxylase, NADPH and NADH are required in the β-ketoacyl-ACP reductase and 2-enoyl-ACP reductase steps, respectively, as well as for desaturation reaction of stearoyl-ACP (Stumpf 1984, 1987). In chloroplasts, ATP and reduced nucleotides are derived from photophosphorylation and NAD(P)H : ferridoxin reductase during photo-synthetic electron transport (Sparace & Kleppinger-Sparace 1993) and during glycolytic metabolism. That illumination is an essential requirement for maximum rates of fatty acid synthesis in isolated chloroplasts which virtually fail to synthesize fatty acids in dark (Smirnov 1960, Nakamura & Yamada 1975, Browse et al. 1981) supports this view point. In non-photosynthetic plastids, ATP and NADH are possibly derived from glycolytic metabolism, whereas NADPH is generated in the pentose phosphate pathway (Sparace & Kleppinger-Sparace 1993). This has been supported by numerous studies which have indicated non-photosynthetic plastids to contain full complements of the enzymes of both these pathways (Entwistle & apRees 1988, Frehner et al. 1990, Journet & Douce 1985, Simcox et al. 1977, Kang & Rawsthorne 1994, Gupta & Singh

Table 1 *Co-factor requirement for in vitro fatty acid biosynthesis from acetate in plastids from several plant tissues*

Cofactor omitted	Pea root plastids[1] % of control activity	Developing castor bean leucoplasts[2]	Daffodil chromoplast[3]	Avocado mesocarp plastids[4]	Developing *Brassica campestris* leucoplasts[5]
ATP	1	62	<1	10	3
CoA	3	91	49	55	10
HCO_3^-	18	-	<<17	33	16
Mg^{2+}	43	76	-	*	4
Mn^{2+}	120	94	*	17	7
Mg^{2+}, Mn^{2+}	15	-	-	-	4
NADH	42	36	92	36	44
NADPH	107	32	94	82	50
NADH, NADPH	19	-	66	-	38

Note: (*) indicates that the factor was not part of the standard reaction mixture. Hyphen (-) denotes not tested. [1]Stahl and Sparace (1991); [2]Miernyk and Dennis (1983); [3]Kleinig and Liedvogel (1978); [4]Weaire and Kekwick (1975); [5]Gupta and Singh (1996a).

1997, Thom et al. 1998). The glycolytic pathway contributes to the production of ATP via the reactions catalyzed by phosphoglycerate kinase and pyruvate kinase, while glyceraldehyde-3-P dehydrogenase provides NADH. NADPH is generated in the pentose phosphate pathway by glucose-6-P dehydro-genase and 6-phosphogluconate dehydrogenase reactions (Sparace & Kleppinger-Sparace 1993). However, a number of studies have also indicated the absence or low activity of one or more of the enzymes of glycolytic and pentose phosphate pathway, precluding the efficient or complete operation of the entire pathways in such non-photosynthetic plastids (Frehner et al. 1990, Emes & Neuhaus 1997). The major enzymes commonly falling in this category include glyceraldehyde-3-P dehydrogenase (Borchert et al. 1993, Trimming & Emes 1993), phospho-glycerate mutase (Borchert et al. 1993) and glucose-6-P dehydrogenase (Frehner et al. 1990, Simcox et al. 1977, Nishimura & Beevers 1979). In view of these observations, it becomes reasonable to believe that non-photosynthetic plastids must interact with extraplastidic (cytosolic) compartment to maximize and maintain high rates of carbon flux through these pathways. This interaction most likely is at the level of phosphate/triose phosphate translocators present in the plastids.

DHAP-Shuttle Mechanism for Generation of Intraplastidic ATP

The phosphate/triose-P translocator, extensively studied in chloroplasts (Flugge et al. 1989, Flugge & Heldt 1991), may form a part of the DHAP shuttle mechanism for promoting ATP synthesis via phosphoglycerate kinase and nucleotide reduction via glyceraldehyde-3-P dehydrogenase in absence of either photophosphorylation as operates in chloroplasts or complete glycolytic metabolism in non-photosynthetic plastids (figure 3). As originally described for chloroplasts (Werdan et al. 1975), DHAP-shuttle requires, in addition to the phosphate translocator, plastidic glycolytic carbon flow from DHAP to 3-PGA via NADP-glyceraldehyde-3-P dehydrogenase and 3-PGA kinase, the latter being responsible for the generation of intraplastidic ATP. The shuttle also requires an exogenous supply of oxaloacetate (OAA), the dicarboxylate translocator, and malate dehydrogenase operating in reverse, in order to regenerate NADP for the dehydrogenas reaction. This shuttle has been known to bypass the light/ATP dependent steps in photosynthetic

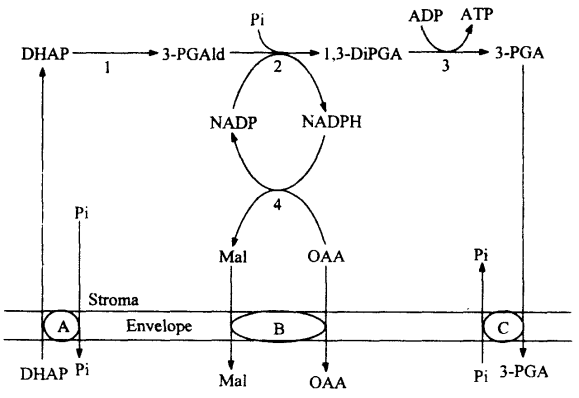

Figure 3 The DHAP shuttle mechanism for the generation of intraplastidic ATP. *3-PGAld*, 3-phosphoglyceraldehyde; *1,3-DiPGA*, 1,3-diphosphoglyceric acid; *3-PGA*, 3-phosphoglyceric acid; *Mal*, malic acid; *OAA*, oxaloacetic acid. 1, dihydroxyacetone phosphate reductase; 2, glyceraldehyde-3-phosphate dehydrogenase; 3, phosphoglycerate kinase; 4, malate dehydrogenase. A & C, triose/phosphate translocator; B, dicarboxylate translocator (*adapted from Werdan et al. 1975*)

CO_2 fixation (Werdan et al. 1975) and fatty acid and sulfolipid biosynthesis (Sauer & Heise 1983, Kleppinger-Sparace & Mudd 1987) in isolated dark-incubated chloroplasts. In recent years, this shuttle has also been shown to promote fatty acid biosynthesis in non-photosynthetic plastids in absence of exogenously supplied ATP (Kleppinger-Sparace et al. 1992, Gupta & Singh 1996c). In pea root leucoplasts, the shuttle could promote upto 40% of the ATP-control activity for fatty acid biosynthesis which has been further improved to the level of the ATP-control when supplemented with ADP. Similarly, in leucoplasts isolated from developing seeds of *Brassica campestris*, the shuttle improved the rate of fatty acid biosynthesis, restoring 50% of the ATP-control activity (Gupta & Singh 1996c).

The ability of DHAP-shuttle to partly substitute for exogenously supplied ATP suggests that a portion of glycolytic metabolism from DHAP to 3-PGA is contained with in the leucoplasts. Further stimulation of fatty acid synthetic activity on addition of ADP suggests that ATP is generated inside of leucoplasts by substrate level phosphorylation at the step catalyzed by phosphoglycerate kinase (Kleppinger-Sparace et al. 1992, Qi et al. 1994). This requires a low ratio of NAD(P)H/NAD(P), which further favours the oxidation of glyceraldehyde-3-P, ensuring high levels of substrate for phosphoglycerate kinase (Werdan et al. 1975). The finding that this enzyme is potentially the second or third most active glycolytic enzyme of non-photosynthetic plastids (Borchert et al. 1993, Trimming & Emes 1993), supports the opinion that in the absence of both externally available ATP and complete plastidic glycolysis, phosphoglycerate kinase might serve as an important source of intraplastidic ATP in non-photosynthetic plastids. Also, the possibility of stimulatory effects of ADP on translocators of the shuttle and the involvement of adenylate kinase cannot be ruled out. In leucoplasts from *Brassica* seeds, addition of 8 mM ATP to the reaction mixture containing shuttle components in appropriate ratio further increased the rate of fatty acid biosynthesis by about 28% compared to that obtained with ATP alone, suggesting that the exogenously supplied ATP and the ATP generated through DHAP shuttle may have additive effect (Gupta & Singh 1996c). This is contrary to the observations made with pea root leucoplasts (Qi et al. 1994) where increase obtained was only to the extent of 6%, indicating little synergism between the effect of externally supplied ATP and the ATP generated internally by the shuttle mechanism. *In vitro* experiments conducted with leucoplasts isolated from pea roots (Qi et al. 1994) and *Brassica* seeds (Gupta & Singh 1996c) to examine the role of DHAP-shuttle in serving as the source of energy for fatty acid synthesis have indicated the optimum concentration of shuttle compoonents (DHAP: OAA:Pi) to be 1:1:2 (Qi et al. 1994, Gupta & Singh 1996c). Further it requires the presence of all shuttle components together for the efficient uptake and metabolism of these compounds as DHAP, G3P and 3-PGA when added alone were ineffective. Contrary to this, Qi et al. (1994) reported optimum concentrations of shuttle components to be 2,2 and 4 mM in pea root leucoplasts. The inhibitory effect of high concentration of shuttle components on fatty acid

synthesis could be attributed to decreased uptake of DHAP by excess of extraplastidic Pi (Flugge & Heldt 1984). Similarly, leakage of glyceraldehyde-3-P may also lead to reduced rates of fatty acid synthesis. Alternatively, as suggested earlier (Emes & Tobin 1993), the phosphorylation and inhibition of regulatory enzymes of the non-photosynthetic plastids might also contribute to decreased rates of fatty acid synthesis. Highest rates of fatty acid synthesis in these experiments were obtained when DHAP was used as the shuttle triose (Qi et al. 1994, Gupta & Singh 1996c). Substitution of DHAP by PEP gave only 60% of the DHAP-stimulated fatty acid synthetic activity. Glyceraldehyde-3-P, 3-PGA and 2-PGA as shuttle trioses were even less effective giving only 36-45% of the DHAP-stimulated shuttle activity.

Other Alternate Sources of Energy

None of the nucleoside triphosphate could substitute ATP in *in vitro* experiments conducted with non-photosynthetic plastids (Kleppinger-Sparace et al. 1992, Gupta & Singh 1996c). Use of ADP alone also resulted in reduced rates of fatty acid synthesis. However, in combination with inorganic phosphate or inorganic pyrophosphate, it improved the rate of fatty acid synthesis. Inorganic phosphate or inorganic pyrophosphate alone again did not serve as an energy source for fatty acid synthesis. AMP along with inorganic pyrophosphate could promote fatty acid synthesis upto 42% of the activity obtained with ATP. These observations point out to the fact that non-photosynthetic plastids have the capacity to generate their own ATP-through the system of adenylate kinase (Murakami & Strotmann 1978). Inorganic pyrophosphate is also known to be involved in a number of energy dependent processes operating in other systems (Stankovic & Walker 1977, Edwards et al. 1985, Hedrich & Schroeder 1989, Xu et al. 1989, Botha & Botha 1990). Besides, some of the glycolytic intermediates can be metabolized in non-photosynthetic plastids to provide energy for fatty acid biosynthesis (Qi et al. 1995). Convincing evidence in support of above have come from *in vitro* studies conducted with chromoplasts of daffodil (Kleinig & Liedvogel 1978), and leucoplasts of castor bean (Boyle et al. 1990), pea roots (Qi et al. 1995) and developing embryos of *Brassica napus* (Kang & Rawsthorne 1996) and *Brassica campestris* (Gupta & Singh 1996b). In daffodil chromoplasts, DHAP, glyceraldehyde-3-P and 3-PGA each could almost completely substitute for ATP. Similarly, 2-PGA and PEP in daffodil chromoplasts and PEP in castor bean leucoplasts gave approximately 2.5 times greater activity than ATP. Similar results were obtained with glucose-6-P (68%) followed by fructose-6-P (50%) and PEP (44%), respectively. With other intermediates as energy sources, the fatty acid synthetic activity ranged from 1 to 38%. The reason for PEP giving higher activity than other triose-P including DHAP could be the ease with which PEP is translocated into the plastids (Borchert et al. 1993). Moreover, only one metabolic reaction is required to produce ATP from PEP by pyruvate kinase. Other metabolites are less efficiently translocated and require multiple enzyme steps before ATP can be synthesized (Qi et al. 1994). On the other hand, hexose-P yields two molecules of triose-P on metabolism through glycolysis, which could ultimately translate into more synthetic activity. These observations are sufficient to suggest that both phosphoglycerate kinase and pyruvate kinase may indeed function to provide the ATP required for fatty acid biosynthesis. Of course, the extent to which they serve this purpose varies from one type of plastid to another (Sparace & Kleppinger-Sparace 1993).

Sources of Reduced Nucleotides

Besides energy, glycolytic and pentose phosphate pathways also generate reduced nucleotides for fatty acid biosynthesis in non-photosynthetic plastids (Emes & Tobin 1993, Emes & Neuhaus 1997). Glyceraldehyde-3-P dehydrogenase, glucose-6-P dehydrogenase and 6-phospho- gluconate dehydrogenase, the key enzymes, participating in this process, are present in these plastids (Kang & Rawsthorne 1994, Gupta & Singh 1997, Thom et al. 1998). Studies to correlate the activities of

these enzymes with promotion of fatty acid biosynthesis in absence of exogenously supplied nucleotides are difficult as plastid envelopes are less permeable to the substrates for these enzymes. Therefore, lysis of plastids is required for getting maximum activities of these enzymes. Unfortunately, this treatment is not conducive to high rates of fatty acid biosynthesis, since intact plastids are essential for achieving such rates (Harwood 1979). This may be the reason, why sorbitol is commonly employed in *in vitro* experiments (Gupta & Singh 1996a).

In *in vitro* experiments with leucoplasts isolated from *Brassica* seeds, exogenous supply of reduced nucleotides was essential for maximum rate of fatty acid synthesis with labelled acetate and glucose (Gupta & Singh 1996b). However, omission of NADH and NADPH individually from the reaction mixture containing labelled glucose resulted only in 46 and 20% loss in synthetic activity, respectively, compared to the corresponding losses of 56 and 50% when labelled acetate was used as the substrate. Omission of both the reduced nucleotides from the reaction mixtures further lowered the rates of fatty acid synthesis. These studies suggest that the metabolism of glucose via plastidic glycolysis and pentose phosphate pathway result in fulfilling the partial requirement of reduced nucleotides (Dennis 1989). This gains further support from the observations of Kang and Rawsthorne (1996), who showed the leucoplasts isolated from developing embryos of oilseed rape to metabolize [1-^{14}C] glucose-6-P simultaneously to starch, fatty acids, and to CO_2 via the oxidative pentose phosphate pathway. They have further shown that during metabolism of glucose-6-P alone, operation of the oxidative pentose phosphate pathway could satisfy the reductant requirement for fatty acid synthesis. However, when pyruvate and glucose-6-P were supplied together, the total calculated demand for NADPH increased substantially while the calculated yield of NADPH from the above pathway remained essentially unchanged. This indicates that other metabolic steps within the leucoplasts may also be contributing to NADPH generation. The fact that pyruvate, can be incorporated into fatty acids at substantial rates even when glucose-6-P is not supplied also provides evidence that the leucoplasts contain other NADPH sources. That could be the activities of glyceraldehyde 3-P dehydrogenase and NADP-malic enzyme, which are now known to be localized in these plastids (Singal et al.1995). Similarly, intraplstidic pyruvate dehydrogenase complex seems to meet the requirement of NADH (Kang & Rawsthorne 1996, Gupta & Singh 1997).

Metabolic Sources of Carbon

The process by which the flow of carbon into the formation of fatty acids is channeled into non-photosynthetic tissues is of great interest. Acetyl CoA represents the ultimate source for fatty acid synthesis, as the formation of malonyl CoA from acetyl CoA and bicarbonate is the first committed step in this synthesis (Stumpf 1984, Harwood 1988, Ohlrogge & Jaworski 1997). Since the plastid envelope is impermeable to acetyl CoA (Brooks & Stumpf 1965), it must be generated within the plastid either by intraplstidic acetyl CoA synthetase (Kuhn et al. 1981) or by pyruvate dehydrogenase complex (Emes & Tobin 1993). Pyruvate dehydrogenase complex, though predominantly mitochondrial (Miernyk et al. 1985, 1987), has also been detected in chloroplasts (Stumpf 1980, Kuhn et al. 1981, Camp & Randall 1985), as well as in non-photosynthetic plastids (Rapp et al. 1987). The activity of pyruvate dehydrogenase complex of chloroplasts is much lower than that of mitochondria (Williams & Randall 1979), necessitating the operation of an alternative pathway for acetyl CoA synthesis. Acetyl CoA formed by pyruvate dehydrogenase complex (Murphy & Stumpf 1981, Liedvogel & Stumpf 1982, Murphy & Walker 1982) is hydrolyzed to acetate by acetyl CoA hydrolase in mitochondria. This free acetate diffuses into the chloroplasts, where it is reconstituted into acetyl CoA by plastidic acetyl CoA synthetase (Kuhn et al. 1981).

Pyruvate as Source of Carbon

Earlier, most *in vitro* studies of fatty acid biosynthesis used acetate as radioactive tracer largely because it is efficiently incorporated into fatty acids. Now it stands well documented that most non-photosynthetic plastids contain their own pyruvate dehydrogenase (Reid et al. 1977, Camp & Randall 1985, Journet & Douce 1985, Liedvogel & Bauerle 1986, Kang & Rawsthorne 1994) and can readily utilize pyruvate for fatty acid biosynthesis (Yamada & Nakamura 1975, Smith et al. 1992, Kang & Rawsthorne 1994), suggesting that pyruvate is physiologically more relevant precursor for fatty acid biosynthesis than free acetate. Furthermore, a number of related studies have indicated that non-photosynthetic plastids contain many glycolytic enzymes to support observed rates of fatty acid biosynthesis (Yamada & Usami 1975, Simcox et al. 1977, Miernyk & Dennis 1983, Journet & Douce 1985, Denyer & Smith 1988, Kang & Rawsthorne 1994). However, as discussed earlier also, in some of the non-photosynthetic plastids one or more enzymes of the glycolytic pathway are absent or have very low activities, thereby, restricting carbon flow through this pathway (Frehner et al. 1990, Borchert et al. 1993, Trimming & Emes 1993). Thus, it is logical to believe that non-photosynthetic plastids rely partly on cytosolic carbon metabolism for their conversion of hexoses to pyruvate via their own glycolytic pathway. Pyruvate dehydrogenase complex coupled with glycolytic metabolism in non-photosynthetic plastids, therefore, represents an efficient and direct source of the acetyl CoA required for fatty acid biosynthesis in these plastids. Alternatively, for non-photosynthetic plastids that have restricted glycolytic carbon flow, pyruvate derived from cytoplasmic glycolysis could also serve as a source of carbon for fatty acid biosynthesis as exogenously supplied radiolabelled pyruvate is readily utilized for fatty acid biosynthesis in isolated plastids.

Malate as Source of Carbon

Smith et al. (1992) examined the incorporation of radio-labelled substrates into fatty acids by leucoplasts isolated from endosperm of developing castor oil seeds. Compared to pyruvate and acetate, exogenous malate was found to support very high rates of fatty acid synthesis in these leucoplast preparations. These workers also detected significant activity of NADP+-malic enzyme in leucoplasts and proposed that malate may be an important alternative carbon substrate for fatty acid synthesis *in vivo*. It was further hypothesized that PEP carboxylase, in concert with cytosolic malate dehydrogenase, converts PEP (derived from imported sucrose via cytosolic glycolysis) into malate which is imported into the leucoplasts for fatty acid biosynthesis. This has gained support from the observations that developing seeds of oil crops contain very high activities of PEP carboxylase (Singal et al. 1987, Sangwan et al. 1992, Podesta & Plaxton 1994a, 1994b, Singal et al. 1995, King et al. 1998) and dark CO_2 fixation and rate of respiration are also very high during active phase of oil synthesis in developing seeds of *Brassica* crops (Singal et al. 1995). Recently, a translocator specific for malate has been identified in leucoplast envelope isolated from developing castor endosperm, that helps in the import of malate in counter exchange of Pi (Eastmond et al. 1997) for fatty acid synthesis in these organelles. This indicates that in the developing seeds of oil seed crops, both the cytosolic and plastidic carbon skeletons and cofactors participate in fatty acid biosynthesis. Recent studies on the photosynthetic characterization of the pods of *Brassica* crops (Sheoran et al. 1991, Singal et al. 1992a, 1992b, 1993. 1995, Dua et al. 1994a, 1994b, Eastmond et al. 1996, King et al. 1998) have indicated that oil synthesis in developing seeds of these crops is supported by pod photosynthesis and dark CO_2 fixation in seeds as the former serves as the source of sucrose and latter as a source of malate.

Hexose and Hexose-P as Sources of Carbon

Under optimum conditions for fatty acid synthesis from acetate (Stahl & Sparace 1991), pea root leucoplasts were shown to readily incorporate radiolabelled pyruvate, glucose and glucose-6-P into fatty acids (Qi et al. 1995). Pyruvate

generally gave higher rates of fatty acid synthesis in these preparations. However, at lower concentrations (0.5-2.0 mM), the rate of fatty acid synthesis was higher from acetate compared to glucose, glucose-6-P and malate. On a nanomolar basis, pyruvate appears to be the preferred *in vitro* carbon donor for fatty acid biosynthesis. However, when one considers that each mole of hexose gives rise to 2 moles of pyruvate, glucose and glucose-6-P can both be considered more effective precursors. In any case, glucose, glucose-6-P and pyruvate can all be metabolized through glycolysis and pyruvate dehydrogenase within pea root leucoplasts to form acetyl CoA, which is then incorporated into fatty acids. Similar observations have also been reported from non-photosynthetic plastids isolated from developing endosperm of *Ricinus communis* (Simcox et al. 1977), cauliflower buds (Journet & Douce 1985), developing embryos of oilseeds rape (Kang & Rawsthorne 1994, 1996) and developing seeds of *Brassica campestris* (Gupta & Singh 1996b). Kang & Rawsthorne (1996), while investigating the partitioning of glucose-6-P to starch, fatty acids and to CO_2 via the oxidative pentose phosphate pathway in leucoplasts isolated from developing embryos of oilseed rape, confirmed the ability of these plastids to utilize this substrate concurrently for these purposes and further calculated the relative fluxes of carbon from glucose-6-P to starch, fatty acids and to CO_2 to be close to 2:1:1. Their studies further revealed weak competition between acetate, pyruvate and glucose-6-P when supplied simultaneously to isolated plastids. This is in contrast with observations made using plastids isolated from a number of other non-photosynthetic tissues (Smith et al. 1992, Fuhrmann et al. 1994, Qi et al. 1995, Gupta & Singh 1996b), where the simultaneous supply of two substrates for fatty acid synthesis had led to a reduction in the rate of incorporation of one substrate relative to when it was supplied alone. From all the above observations, it can safely be interpreted that there is complete operation of glycolytic pathway in most of the non-photosynthetic plastids, which would lead to the production of pyruvate from the imported sugar. In cases, where one or the other enzyme of this pathway is lacking, the two compartments (cytosolic and plastidic) can interact through the operation of the translocators located in the inner envelope of the plastids. These translocators help in the exchange of a number of metabolites of glycolytic pathway across the envelope of the non-photosynthetic plastids and ensure the efficiency of the metabolic processes localized in these organelles.

Metabolite Exchange Through Plastid Envelope

The double membrane structure surrounding the plastids ensures selective permeability of metabolites to these organelles (Emes & Neuhaus 1997, Emes & Tobin 1993). The outer membrane contains porins, which allow the free permeability of hydrophilic molecules with a molecular mass of upto 10 kDa (Flugge & Benz 1984, Fischer et al. 1994a) facilitating selective targetting of proteins to the plastids (Gray & Row 1995), whereas the inner membrane is the site for selective permeability of small molecules through the translocators across the membrane (Emes & Neuhaus 1997). The above phenomenon of selective permeability offers the means by which these plastids control their metabolism, and also a medium through which the metabolic/ bioenergetic status of these organelles may be communicated to the rest of the cell (Emes & Tobin 1993). Chloroplasts, which are best studied plastids in this regard, counter exchange inorganic phosphate and triose phosphates, dicarboxylates and amino acids, and amides (Heldt & Flugge 1987, 1992, Flugge & Heldt 1991). This serves as a means of exporting photosynthate to the cytoplasm, controlling partitioning between sucrose and starch, exporting products of nitrogen assimilation, and indirectly moving reducing power and energy between the two compartments.

Transport of Phosphorylated Metabolites

The best studied translocator is the triose phosphate translocator (TPT) of the inner chloroplast envelope (Flugge et al. 1989) which is the largest

protein fraction of the chloroplast envelope and may account for upto 15% of the envelope protein. The translocator, consisting of a dimer of two identical polypeptide chains with molecular mass of 29 kDa each, catalyzes the counter-exchange of inorganic phosphate (Pi), triose-P and 3-PGA, which compete with each other for a common binding site. An analogous translocator in non-photosynthetic tissues was first demonstrated in amyloplasts from pea roots (Emes & Traska 1987) and this translocator catalyzes the counter exchange of glu-6-P with Pi (Borchert et al. 1989, Kammerer et al. 1998). The hexose phosphate translocator (HPT) from pea root plastids did not appear to import glu-1-P and had an apparent Km of 0.54 mM for glu-6-P. Similar translocators have been observed in plastids isolated from cauliflower buds (Mohlmann et al. 1995), pea embryo amyloplasts (Hill & Smith 1995) and sweet pepper chromoplasts (Thom et al. 1998). Substrate kinetic studies using either purified plastids or plastid membrane proteins reconstituted into liposomes, have indicated HPT to transport glu-6-P, glu-1-P, DHAP, PEP, PGA, and Pi (Borchert et al. 1993, Mohlmann et al. 1995, Quick & Neuhaus 1996, Tetlow et al. 1996). However, whether these plastids contain a single carbon transporter with broader specificity, or more than one, with each having different substrate affinities, is not yet clear. Recently, Fischer et al. (1997) have identified a translocator from maize kernel amyloplasts, which showed marked preference for PEP and 2-PGA over triose phosphates, and had no activity towards either glu-6-P or glu-1-P, indicating that there are at least two types of phosphate translocators in amyloplast envelopes and possibly more. The PEP translocator may have significance for the synthesis of aromatic amino acids, which needs import of PEP, thus bypassing some of the missing reactions of glycolysis (catalyzed by phosphoglyceromutase and enolase) in some of the non-photosynthetic plastids (Trimming & Emes 1993, Miernyk & Dennis 1992). A specific transport protein allowing the import of glu-1-P in counter exchange for other phosphorylated intermediates and triose-P has also been identified in a number of heterotrophic plastids including amyloplasts from cauliflower buds (Mohlamann et al. 1995), chromoplasts from tomato fruits (Schunemann & Borchert 1994) and amyloplasts of wheat endosperm (Tetlow et al. 1996). However, there is conflicting evidence on the transport of glu-1-P into potato amyloplasts (Kosegarten & Mengel 1994, Schott et al. 1995, Naeem et al. 1997). Even the glu-6-P transporter of sweet pepper chromoplasts does not import glu-1-P (Thom et al. 1998). Hence, in amyloplasts, there may be diverse hexose phosphate translocators, some able to catalyze counter exchange involving glu-6-P only, others use glu-1-P or glu-6-P, and some importing glu-1-P without counter exchange with any substrate (Mohlmann et al. 1995). The amyloplasts from cauliflower buds have also been shown to possess a specific transport protein for glucose (Mohlmann et al. 1995). However, this transporter has been suggested to be involved in export of glucose during starch degradation (Batz et al. 1994, Neuhaus et al. 1995). At present there are only a few examples of fully characterized plastidic sugar transporters.

Transport of Malate

In a recent study, Eastmond et al. (1997) have proposed that leucoplasts from developing castor endosperm contain a malate/Pi translocator that imports malate in strict counter exchange for Pi. Earlier studies (Lehner & Heldt 1978, Day & Hatch 1981, Proudlove & Thurmann 1981) on malate transport in chloroplasts of C_3 and C_4 plants had shown that Pi is not a substrate of either the dicarboxylate or the 2-oxo-glutarate transporters. Further, 1:1 stoichiometry of malate/Pi exchange, in conjunction with the ability of both Pi and n-butyl malonate to abolish more than 70% of the rate of malate uptake, suggests that the Pi/malate- exchange translocator is likely to be the major activity associated with malate uptake by the leucoplasts. The small inhibition of malate uptake by glutamate, malonate, succinate, and 2-oxo-glutarate indicates that other

dicarboxylate transporters may also be present in the leucoplast inner membrane. Alternatively, some of these metabolites may also be transported by the leucoplast Pi/malate- exchange translocator. The presence of malate/Pi translocator in the leucoplast supports the hypothesis advocated earlier by Smith et al. (1992), where malate has been proposed to be an important alternative carbon substrate for fatty acid synthesis *in vivo*. That the rate of malate transport was more than 1.5-fold in excess of the rate of incorporation of carbon from malate into fatty acids by isolated leucoplasts at 5 mM substrate concentration has also been indicated by these workers. This concentration is saturating for both malate uptake and the incorporation of carbon from malate into fatty acids. Both malate uptake and malate-dependent fatty acid synthesis were strongly inhibited by *n*-butyl malonate (Eastmond et al. 1997), suggesting that the function of the malate/Pi translocator is to import malate across the leucoplast envelope for fatty acid synthesis. The incorporation of carbon from malate into fatty acids results in the generation of Pi within the leucoplast. This Pi is released from ATP during the conversion of acetyl CoA to malonyl-CoA by acetyl-CoA carboxylase, and is exported in counter exchange for malate.

Transport of Adenylates

Since non-photosynthetic plastids require the supply of ATP and reducing power to sustain their biosynthetic activities (Tetlow et al. 1994, Hill & Smith 1991, Kang & Rawsthorne 1994, 1996, Mohlmann et al. 1994, Singh 1992, 1993, Gupta & Singh 1996a, 1996b), so it necessitates the presence of mechanisms for the import of either ATP, and/or adenylates to be used for ATP generation within the organelle by substrate level phosphorylation. This has been supported by the observations where triose phosphate shuttle was shown to meet the partial requirement of ATP for fatty acid biosynthesis from acetate in isolated leucoplasts from pea roots (Qi et al 1994) and developing seeds of *Brassica* (Gupta & Singh 1996c), suggesting that whether ADP is phosphorylated within the organelle or enters as ATP, adenylates can be transported (Schunemann et al. 1993, Pozueta-Romero et al. 1991). Further experiments with pea root plastids have indicated that the plastidic adenylate translocator has a significantly different structure than that of its mitochondrial counterpart (Kampfenkel et al. 1995).

Model of Carbon Flow for Fatty Acid Biosynthesis

Based on the informations contained in all previous sections of this review, a model (figure 4) has been proposed for fatty acid synthesis in leucoplasts of non-photosynthetic tissues (Gupta & Singh 1997). According to this model, sucrose (the form in which assimilates move and serve as the source of carbon) on entering the cytosol is hydrolyzed either by sucrose synthase (cleavage) and/or invertase. Both these enzymes are now known to be present in non-photosynthetic plastids (Singh 1992). Part of hexoses/ hexoses-P produced in cytosol are translocated into plastids through the phosphate translocators specific for hexoses, mainly glucose (Batz et al. 1993, 1994, Neuhaus et al. 1995) and hexose-P (glucose-6-P) (Hill & Smith 1995, Mohlmann et al. 1995), and enter into glycolytic and oxidative pentose phosphate pathways independently in both the compartments (cytosolic and plastidic), yielding pyruvate as the end product of glycolysis and producing NADPH through the reactions of oxidative pentose phosphate pathway. Some of the intermediates of glycolysis such as DHAP, 3-PGA, 2-PGA, PEP can also be taken up into leucoplasts through phosphate translocators in exchange for Pi (Emes & Neuhaus 1997). Frehner et al (1990) have discussed the possibility of pyruvate transport also in non-photosynthetic plastids particularly in view of the fact that glycolysis in many of these plastids has been shown to be incomplete, and the pyruvate is needed for the reaction of pyruvate dehydrogenase complex during fatty acid biosynthesis. PEP carboxylase present in cytosolic

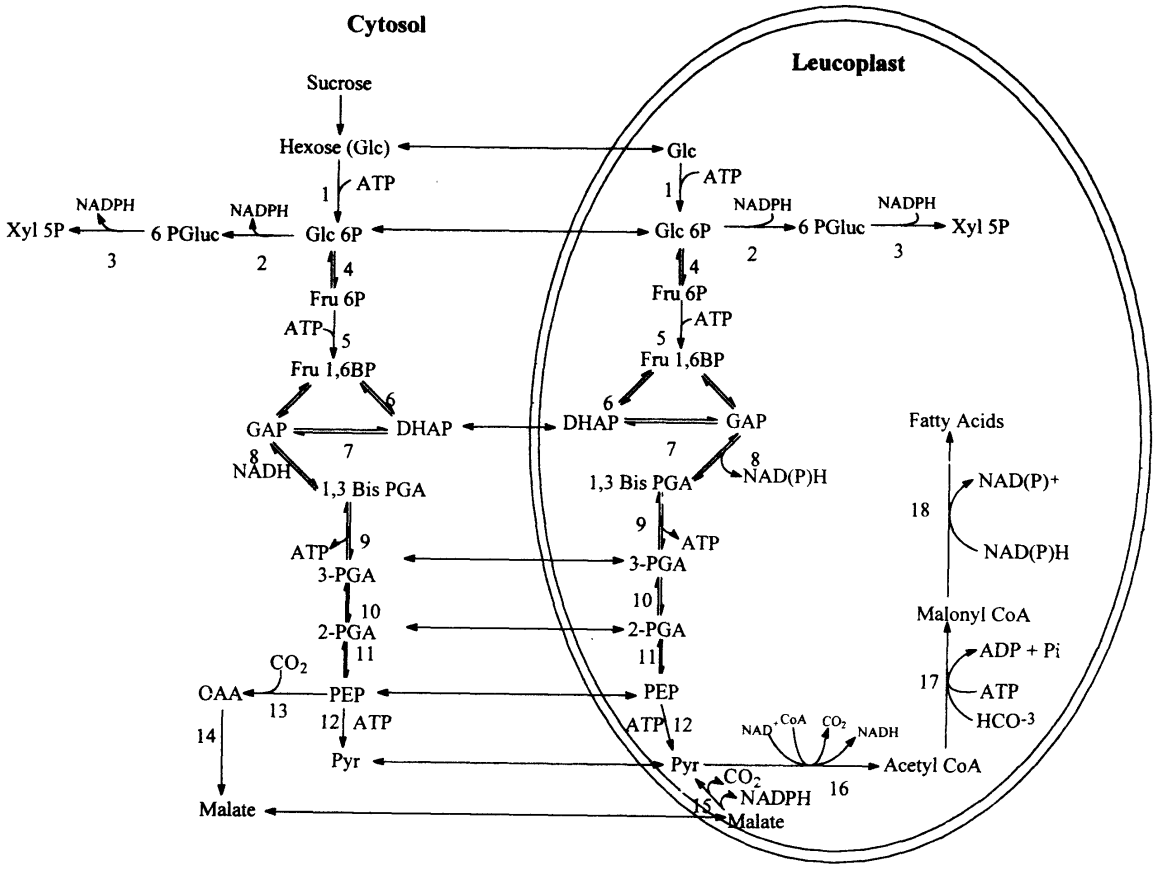

Figure 4 A model depicting the enzymatic steps and exchange of carbon in the cytosolic and leucoplastic compartments of developing embryos of oil seed crops. [1, hexokinase; 2, glc-6-P dehydrogenase; 3, 6-P-gluc dehydrogenase; 4, hexose phosphate isomerase; 5, phosphofructokinase; 6, aldolase; 7, triose phosphate isomerase; 8, glyceraldehyde-3P dehydrogenase; 9, 3-PGA kinase; 10, PGA mutase; 11, enolase; 12, pyruvate kinase; 13, PEP carboxylase; 14, malate dehydrogenase; 15, NADP-malic enzyme; 16, pyruvate dehydrogenase complex; 17, acetyl CoA carboxylase; 18, fatty acid synthetase complex].

fraction of developing seeds of oil bearing crops fixes respired CO_2 to yield OAA which is ultimately reduced to malate (Singal et al. 1987, 1995). Thus, malate, in turn is translocated into leucoplasts through a malate translocator in counter-exchange for Pi (Eastmond et al. 1997) or through translocators meant for dicarboxylates (Emes & Tobin 1993), and is converted to pyruvate in plastids via NADP-malic enzyme which is known to be localized in the leucoplasts (Singal et al. 1995, Kang & Rawsthorne 1994). Besides, this reaction also generates NADPH needed for fatty acid biosynthesis. However, for this synthesis, Kang & Rawsthorne (1996) have recently shown sufficient production of NADPH through oxidative pentose phosphate pathway in leucoplasts from developing rape embryos. The imported pyruvate joins the pyruvate pool generated by plastidic pyruvate kinase (Plaxton 1991, Plaxton 1996, Podesta & Plaxton 1994a, 1994b, Singh et al. 1998) and gets converted to acetyl CoA via pyruvate dehydrogenase complex. Acetyl CoA, thus generated, serves as the source of carbon for fatty acid synthesis in leucoplasts of developing seeds of oil crops. The reported incorporation of label into fatty acids from acetate, DHAP, pyruvate, malate, glucose and glu-6-P (Kleppinger-Sparace et al. 1992, Qi et al. 1994, Kang & Rawsthorne 1994, 1996, Gupta & Singh 1996b) can very well be explained by the model proposed here.

Conclusions

Since non-photosynthetic plastids in crop plants are involved in the biosynthesis of seed reserves, so an understanding of their functioning at molecular level will go a long way in bringing about metabolic manipulations through genetic engineering in these plastids. This is a long term aim to enhance the capacity of such plastids to synthesize more reserve material. Though attempts made in this direction during the last decade have improved our knowledge of the metabolism which occur in non-photosynthetic plastids, yet many important details remain poorly understood. The nature of carbon translocated and utilized for various biosynthetic activities has been well recognized now. However, the information on the molecular nature of the various transporters located in the inner envelope of these plastids and the pattern of expression of these translocators at the cellular level is still scanty. There may also be developmental changes in the expression of metabolic pathways inside the plastids and *in vitro* studies carried out with isolated plastids suffer from many such limitations. It is, therefore, essential to obtain information on the role of these plastids in adjusting their metabolism with changing developmental stage of a tissue. Use of transgenic plants in which cell specific isoforms of a protein can be down-regulated by use of antisense technique, could help us in delineating the role of various enzymes in different metabolic pathways. Very little information is available on the subcellular compartmentation of metabolites *in vivo* and the application of non-aqueous techniques to non-green tissues has met with only limited success. Detailed investigations are also needed in this direction so that a complete vision on the regulatory aspects of different metabolic pathways could be obtained. Such vital information may eventually help us in developing crop cultivars with better productivity.

References

Batz O, Scheibe R and Neuhaus H E 1993 Identification of the putative hexose phosphate translocator of amyloplasts from cauliflower buds; *Biochem. J.* **294** 15-17

_____, _____ and_____1994 Glucose and ADP-glucose dependent starch synthesis in isolated cauliflower bud amyloplasts. Analysis of the interaction of various potential precursors; *Biochim. Biophys. Acta* **1200** 148-154

Borchert S, Grosse H and Heldt H W 1989 Specific transport of inorganic phosphate, glucose-6-P, DHAP and 3-PGA into amyloplasts; *FEBS Lett.* **253** 183-186

_____, Harborth J, Schunemann D, Hoferichter P and Heldt H W 1993 Studies of the capacities and transport properties of pea root plastids; *Plant Physiol.* **101** 303-312

Botha A M and Botha F C 1990 Control of pyrophosphate: D- fructose-6-P 1-phosphotransferase activity in the cotyledons of *Citrillus lanatus*; *Plant Physiol.* **93** 683-688

Boyle S A, Hemmingsen S M and Dennis D T 1990 Energy requirement for the import of protein into plastids from developing endosperm of Ricinus communis L; *Planta* **177** 359-366

Brooks J L and Stumpf P K 1965 A soluble fatty acid synthesizing system for lettuce chloroplasts; *Biochim. Biophys. Acta* **98** 213-216

Browse J 1997 Synthesis and storage of fatty acids in *Cellular and Molecular Biology of Plant Seed Development*, pp 407-440 eds B A Larkins and I K Vasil (Dordrecht: Kluwer Academic Publishers)

_____ and Slack C R 1985 Fatty acid synthesis in plastids from maturing safflower and linseed cotyledons; *Planta* **166** 74-80

_____ and Somerville C R 1994 Glycerolipids in *Arabidopsis* pp 881-912 eds E M Meyerwitz and C R Somerville (Plainview, NY: Cold Spring Harbor)

_____ Roughan P G and Slack C R 1981 Light control of fatty acid synthesis and diurnal fluctuations of fatty acid compositions of leaves; *Biochem. J.* **196** 347-354

Camp P J and Randall D D 1985 Purification and characterization of pea chloroplast pyruvate dehydrogenase complex; *Plant Physiol.* **77** 571-577

Day D A and Hatch M D 1981 Transport of 3-PGA, PEP and Pi in maize mesophyll chloroplasts, and the effect of 3-PGA on malate and PEP production; *Arch. Biochem. Biophys.* **211** 743-749

Dennis D T 1989 Fatty acid biosynthesis in plastids; in *Physiology, Biochemistry and Genetics of Non-green Plastids* pp 120-129 eds C D Boyer, J C Shannon and R C Hardison (Rockwille: American Society of Plant Physiologists)

_____, Blakeley S and Carlisle S 1991 in *Compartmentation of Plant Metabolism in Non-photosynthetic Tissues* Vol 42 pp 77- 94 ed M J Emes SEB Seminar Series (Cambridge: Cambridge University Press)

Denyer K and Smith A M 1988 The capacity of plastids from developing pea cotyledons to synthesize acetyl CoA; *Planta* **173** 172-182

Dua A, Talwar G and Singh R 1994a Changes in structure and function of photosynthetic apparatus of *Brassica* pods during seed maturation; *Indian J. Biochem. Biophys.* **31** 171-176

_____, _____, Singal H R and Singh R 1994b CO_2 exchange, primary photochemical reactions and enzymes of photosynthetic carbon reduction cycle in Brassica pods during water sterss and recovery; *Photosynthetica* **30** 261-268

Eastmond P J, Kolaena L and Rawsthorne S 1996 Photosynthetic characteristics of developing embryos of oil seed rape; *J. Exp. Bot.* **47** 1763-1769

_____, Dennis D T and Rawsthorne S 1997 Evidence that a malate/inorganic phosphate exchange translocator imports carbon across the leucoplast envelope for fatty acid synthesis in developing castor seed endosperm; *Plant Physiol.* **114** 851-856

Edwards G E, Nakamato H, Burnell J N and Hatch M D 1985 Pyruvate, Pi dikinase and NADP-malate dehydrogenase in C4 photosynthesis; Properties and mechanisms of light/dark regulation; *Annu. Rev. Plant Physiol.* **36** 255-286

Emes M J and Fowler M W 1979 The intracellular location of the enzymes of nitrate assimilation in the apices of seedling pea roots; *Planta* **145** 287-292

_____ and _____ 1983 The supply of reducing power for nitrite reduction in plastids of seedling pea roots (Pisum sativum L); *Planta* **158** 97-102

_____ and Traska A 1987 Uptake of inorganic phosphate by plastids purified from the roots of *Pisum sativum* L; *J. Exp. Bot.* **38** 1781-1788

_____ and Tobin A K 1993 Control of metabolism and development in higher plant plastids; *Int. Rev. Cytol.* **145** 149-216

_____ and Neuhaus H E 1997 Metabolism and transport in non- photosynthetic plastids; *J. Exp. Bot.* **48** 1995-2005

Entwistle G and apRees T 1988 Enzymic capacities of amyloplasts from wheat *(Triticum aestivum)* endosperm; *Biochem. J.* **255** 391-396

Fischer K, Weber A, Brink S, Arbinger B, Schuenemann S, Borchert S, Heldt H W, Popp B, Benze R, Link T A, Eckerskorn C and Flugge U I 1994a Porins from plants. Molecular characterization of the two members of the porin family; *J. Biol. Chem.* **269** 25754-25760

_____, Kammerer B, Gutensohn M, Arbinger B, Weber A, Hausler R and Flugge U I 1997 A new class of plastidic phosphate translocator: a putative link between primary and secondary metabolism by PEP/phosphate antiporter; *Plant Cell* **9** 453-462

Flugge U I and Benz R 1984 Pore forming activity in the outer membrane of the chloroplast envelope; *FEBS Lett.* **169** 85-89

_____ and Heldt H W 1984 The phosphate-triose phosphate- phosphoglycerate translocator of chloroplasts; *Trends Biochem. Sci.* **9** 530-533

_____ and _____ 1991 Metabolite translocators of the chloroplast envelope; *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **42** 129-144

_____, Fischer K, Gross A, Sebald W, Lottspeich F and Eckerskorn C 1989 The triose phosphate - 3-phosphoglycerate- phosphate translocator from spinach chloroplasts: nucleotide sequence of a full length cDNA clone and import of the *in vitro* synthesized precursor protein into chloroplasts; *EMBO J.* **8** 39-46

Frehner M, Pozueta-Romero J and Akazawa T 1990 Enzyme sets of glycolysis, gluconeogenesis, and oxidative pentose phosphate pathway are not complete in nongreen highly purified amyloplasts of Sycamore (Acer pseudoplatanus L) cell suspension cultures; *Plant Physiol.* **94** 538-544

Fuhrmann J, Johnen T and Heise K P 1994 Compartmentation of fatty acid metabolism in zygotic rape embryos; *J. Plant Physiol* **143** 565-569

Gray J C and Row P E 1995 Protein translocation across chloroplast envelope membranes; *Trends Cell Biol.* **5** 243-247

Gupta R and Singh R 1996a Fatty acid synthesis in leucoplasts isolated from developing seeds of *Brassica campestris*; *J Plant Biochem. Biotech.* **5** 127-130

_____ and _____ 1996b Fatty acid synthesis by isolated leucoplasts from developing *Brassica* seeds: Role of glycolytic intermediates as the source of carbon and energy; *Indian J. Biochem. Biophys.* **33** 478-483

—— and —— 1996c Fatty acid synthesis by isolated leucoplasts from developing *Brassica* seeds: Role of nucleoside triphosphates and DHAP-shuttle as the source of energy; *J. BioSci.* **21** 819-826

—— and —— 1997 Enzymes of glycolytic and pentose phosphate pathways in cytosolic and leucoplastic fractions of developing seeds of *Brassica campestris*; *Indian J. Biochem. Biophys.* **34** 288-295

Harwood J L 1979 The synthesis of acyl lipids in plant tissues; *Prog. Lipid Res.* **18** 55-86

—— 1988 Fatty acid metabolism; *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **39** 101-138

—— 1996 Recent advances in the biosynthesis of plant fatty acids; *Biochim. Biophys. Acta* **301** 7-56

Hedrich R and Schroeder J I 1989 The physiology of ion channels and electrogenic pumps in higher plants; *Annu Rev. Plant Physiol. Plant Mol. Biol.***40** 539-569

Hedlt H W 1997 Glycerolipids; in *Plant Biochemistry and Molecular Biology* pp 317-351 H W Heldt (Oxford: Oxford University Press)

—— and Flugge U I 1992 Metabolite transport in plant cells; in *Plant Organelles, Compartmentation of Metabolism in Photosynthetic Cells* pp 21-47 ed. A K Tobin (Cambridge: Cambridge Unversity Press)

—— and —— 1987 Subcellular transport of metabolites in plant cells; in *The Biochemistry of Plants* Vol 12 pp 49-85 eds P K Stumpf and E E Conn (Orlando FL: Academic Press)

Hill L M and Smith A M 1991 Evidence that glucose-6-P is imported as the substrate for starch synthesis by plastids of developing pea embryos; *Planta* **185** 91-96

—— and —— 1995 Coupled movement of glucose-6 P and triose-P across the envelopes of plastids from developing embryos of pea (*Pisum sativum* L); *J. Plant Physiol.* **146** 411-417

Journet E P and Douce T 1985 Enzyme capacities of purified cauliflower bud plastids for lipid synthesis and carbohydrate metabolism; *Plant Physiol.* **79** 458-467

Kammerer B, Fischer K, Hilpert B, Schubert S, Gutensohn M, Weber A and Flugge U I 1998 Molecular characterization of a carbon translocator in plastids from heterotrophic tissues: the glucose-6-P/phosphate antiporter; *Plant Cell* **10** 105-112

Kampfenkel K, Mohlmann T, Batz O, Van Montagu M, Inze D and Neuhaus H E 1995 Molecular characterization of an *Arabidopsis thaliana* cDNA encoding for a novel putative adenylate transporter of higher plants; *FEBS Lett.* **374** 351- 355

Kang F and Rawsthorne S 1994 Starch and fatty acid synthesis in plastids from developing embryos from oil seed rape (Brassica napus L); *Plant J* **6** 795-805

—— and —— 1996 Metabolism of glucose-6-P and utilization of multiple metabolites for fatty acid synthesis by plastids from developing oil seed rape embryos; *Planta* **199** 321-327

King S P, Badger M R and Furbank R T 1998 CO2 refixation capacities of developing canola seeds and silique walls; *Aust. J. Plant Physiol.* **25** 377-386

Kinney A J 1994 Genetic modification of the storage lipids of plants; *Curr. Opin. Biotech.* **5** 144-151

Kleinig H and Liedvogel B 1978 Fatty acid synthesis by isolated chromoplasts from the daffodil. (^{14}C) acetate incorporation and distribution of labeled acids; *Eur. J. Biochem.* 83 499- 505

—— and—— 1980 Fatty acid synthesis by isolated chromoplasts from daffodil. Energy sources and distribution patterns of acids; *Planta* **150** 166-169

Kleppinger-Sparace K F and Mudd J B 1987 Biosynthesis of sulfoquinovosyl-diacylglycerol in higher plants: The incorporation of $^{35}SO_4$ by intact chloroplasts in darkness; *Plant Physiol.* **84** 682-687

—— , Stahl R J and Sparâce S A 1992 Energy requirements for fatty acid and glycerolipid biosynthesis from acetate by isolated pea root plastids; *Plant Physiol.* **98** 723-727

Kosegarten H and Mengel K 1994 Evidence for a glucose-1-P translocator in storage tissue amyloplasts of potato (*Solanum tuberosum*) suspension-cultured cells; *Physiol. Plant.* **91** 111-120

Kuhn D N, Knauf M and Stumpf P K 1981 Subcellular localization of acetyl CoA synthetase in leaf protoplasts of *Spinacia oleracea*; *Arch. Biochem. Biophys.* **209** 441-450

Lehner K and Heldt H W 1978 Dicarboxylate transport across the inner membrane of the chloroplast envelope; *Biochim. Biophys. Acta* **501** 531-544

Liedvogel B and Stumpf P K 1982 Origin of acetate in spinach leaf cells; *Plant Physiol.* **69** 897-903

—— and Bauerle R 1986 Fatty acid synthesis in chloroplasts from mustard (*Sinabis alba* L) cotyledons: formation of acetyl CoA by intraplastid glycolytic enzymes and a pyruvate dehydrogenase complex; *Planta* **169** 481-489

Miernyk J A and Dennis D T 1983 The incorporation of glycolytic intermediates into lipids by plastids isolated from the developing endosperm of castor oil seed (*Ricinus communis* L); *J. Exp. Bot.* **34** 712-718

------- and ----- 1992 A developmental analysis of the enolase isozymes from *Ricinus communis*; *Plant Physiol.* **99** 748-750

_____, Camp R J and Randall D D 1985 Regulation of plant pyruvate dehydrogenase complexes; *Curr Top. Plant Biochem. Physiol.* **4** 175-190

_____, Rapp B J, David N R and Randall D D 1987 in *Plant Mitochondria* pp 189-197 eds A L Moore and B R Beechev (London: Plenum)

Mohlmann T, Scheibe R and Neuhaus H E 1994 Interaction between fatty acid and starch synthesis in isolated amyloplasts from cauliflower buds; *Planta* **194** 492- 497

_____, Batz O, Maa B U and Neuhaus H E 1995 Analysis of carbohydrate transport across the envelope of isolated cauliflower bud amyloplasts; *Biochem. J.* **307** 521-526

Murakami S and Strotmann H 1978 Adenylate kinase bound to the envelope membranes of spinach chloroplasts; *Arch. Biochem. Biophys.* **185** 30-38

Murphy D J 1994 Biogenesis, function and biotechnology of plant storage lipids; *Prog. Lipid Res.* **33** 71-85

_____ and Stumpf P K 1981 The origin of chloroplast acetyl CoA; *Arch. Biochem. Biophys.* **212** 730-739

_____ and Walker D A 1982 The properties of transketolase from photosynthetic tissue; *Planta* **156** 84-88

Naeem M, Tetlow I J and Emes M J 1997 Starch synthesis in amyloplasts purified from developing potato tubers; *Plant J.* **11** 101-108

Nakamura Y and Yamada M 1975 Fatty acid synthesis by spinach chloroplasts. III. Relationship between fatty acid synthesis and photophosphorylation; *Plant Cell Physiol.* **16** 163-174

Neuhaus H E, Henrichs G and Scheibe R 1995 Starch degradation in intact amyloplasts isolated from cauliflower floral buds (*Brassica oleracea* L); *Planta* **195** 496-504

Nishimura M and Beevers H 1979 Subcellular distribution of gluconeogenetic enzymes in germinating castor bean endosperm; *Plant Physiol.* **64** 31-37

Nothelfer H G, Barckhaus R H and Spener F 1977 Localization and characterization of fatty acid synthesizing system in cells of *Glycine max* suspension cultures; *Biochim. Biophys. Acta* **489** 370-380

Ohlrogge J and Browse J 1995 Lipid biosynthesis; *Plant Cell* **7** 957-970

------ and Jaworski J G 1997 Regulation of fatty acid synthesis; *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **48** 109-136

Oji Y, Watanable M, Wakiuchi N and Okamato S 1985 Nitrite reduction in barley-root plastids: dependence on NADPH coupled with glucose-6-P and 6-phosphogluconate dehydrogenase and possible involvement of an electron carrier and a diaphorase; *Planta* **165** 85-90

Page R A, Okada S and Harwood J L 1994 Acetyl CoA carboxylase exerts strong flux control over lipid synthesis in plants; *Biochim. Biophys. Acta* **1210** 369-372

Plaxton W C 1991 Leucoplast pyruvate kinase from developing castor oil seeds; *Plant Physiol.* **97** 1334-1338

_____ 1996 The organization and regulation of plant glycolysis; *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47** 185-214

Podesta F E and Plaxton W C 1994a Regulation of carbon metabolism in germinating *Ricinus communis* cotyledons. I Developmental changes in activity levels and molecular structure of glycolytic enzymes during germination; *Planta* **194** 381-387

_____ and _____ 1994b Regulation of cytosolic carbon metabolism in germinating Ricinus communis cotyledons. II Properties of PEP carboxylase and cytosolic pyruvate kinase associated with the regulation of glycolysis and nitrogen assimilation; *Planta* **194** 381-387

Proudlove M O and Thurman D A 1981 The uptake of 2-oxoglutarate and pyruvate by isolated pea chloroplasts; *New Phytol.* **88** 255- 264

Pozueta-Romero J, Frehner M, Viale A M and Akazawa T 1991 Direct transfer of ADP-glucose by an adenylate translocator is linked to starch biosynthesis in amyloplasts; *Proc. Natl. Acad. Sci. USA* **88** 5769-5773

Qi Q, Kleppinger-Sparace K F and Sparace S A 1994 The role of the triose phosphate shuttle and glycolytic intermediates in fatty acid and glycerolipid biosynthesis in pea root plastids; *Planta* **194** 193-199

_____, _____ and _____1995 The utilization of glycolytic intermediates as precursors of fatty acid biosynthesis by pea root plastids; *Plant Physiol.* **107** 413-419

Quick W P and Neuhaus H E 1996 Evidence for two types of phosphate translocators in pepper (*Capsicum annuum* L) fruit chromoplasts; *Biochem. J.* **320** 7-10

Rapp B J, Miernyk J A and Randall D D 1987 Pyruvate dehydrogenase complexes from *Ricinus communis* endosperm; *J. Plant Physiol.* **127** 293-306

Reid E E, Thompson P, Lyttle R C and Dennis D T 1977 Pyruvate dehydrogenase complex from higher plant mitochondria and proplastids; *Plant Physiol.* **59** 842-848

Sangwan R S, Singh N and Plaxton W C 1992 PEP carboxylase activity and concentration in the endosperm of developing and germinating castor oil seeds; *Plant Physiol.* **99** 445-449

Sauer A and Heise K P 1983 On the light dependence of fatty acid synthesis in spinach chloroplasts; *Plant Physiol.* **73** 11-15

Schott K, Borchert S, Muller-Rober B and Heldt H W 1995 Transport of inorganic phophate and C_3 and C_6-sugar phosphates across the envelope membranes of potato tuber amyloplasts; *Planta* **196** 647-652

Schunemann D and Borchert S 1994 Specific transport of inorganic phosphate and C_3 and C_6 sugar phosphates across the envelope membranes of tomato (*Lycopersicon esculentum*) leaf chloroplasts, tomato fruit chloroplasts and fruit chromoplasts; *Botanica Acta* **107** 461-467

_____, _____ , Flugge, U I and Heldt H W 1993 ATP/ADP translocator from pea root plastids. Comparison with translocators from spinach chloroplasts and pea root mitochondria; *Plant Physiol.* 103 131-137

Sheoran I S, Sawhney V, Babbar S and Singh R 1991 In vitro fixation of CO_2 by attached pods of *Brassica campestris* L; *Ann. Bot.* **67** 425-428

Simcox P D, Reid E E, Canvin D T and Dennis D T 1977 Enzymes of glycolytic and pentose phosphate pathways in proplastids of *Ricinus communis*; *Plant Physiol.* **59** 1228-1232

Singal H R, Sheoran I S and Singh R 1987 Photosynthetic carbon fixation characteristics of fruiting structures of *Brassica campestris*; *Plant Physiol.* **83** 1043-1047

_____, _____ and_____1992a Photosynthetic contribution of pods towards seed yield in *Brassica*; *Proc. Indian Natl. Sci. Acad.* **B58** 365-370

_____, Laura J S and Singh R 1992b Ontogenic changes in photosynthetic carbon reduction cycle metabolites and enzymes of sucrose synthesis in *Brassica campestris* pods; *Photosynthetica* **26** 463-468

_____, _____ and _____1993 Photosynthetic carbon reduction cycle metabolites and enzymes of sucrose and starch biosynthesis in developing *Brassica* pods; *Indian J. Biochem. Biophys.* **30** 270-276

_____, Talwar G, Dua A and Singh R 1995 Pod photosynthesis and seed dark CO_2 fixation support oil synthesis in developing Brassica seeds; *J. BioSci* **20** 49-58

Singh R 1992 Carbon source for starch biosynthesis in amyloplasts of plant storage tissues; *Indian J. Biochem. Biophys.* **29** 1-8

_____ 1993 Photosynthetic characteristics of fruiting structures of cultivated crops; in *Photoreactions to Plant Productivity* pp 389-415 eds Y P Abrol, P Mohanty and Govindjee (The Netherland : Kluwer Academic Publishers)

Singh D K, Malhotra P Sarla and Singh R 1998 Purification and characterization of plastidic pyruvate kinase from developing seeds of *Brassica campestris* L; *Indian J. Biochem. Biophys.* **35** in press

Smith R G, Gautheir D A, Dennis D T and Turpin D H 1992 Malate and pyruvate dependent fatty acid synthesis in leucoplasts from developing castor endosperm; *Plant Physiol.* **98** 1233-1238

Smirnov B P 1960 The biosynthesis of higher acids from acetate in isolated chloroplasts of *Spinacia oleracea* leaves; *Biokhimiya* **25** 545-555

Sparace S A ans Kleppinger-Sparace K F 1993 Metabolism in non- photosynthetic, non-oil seed tissues; in *Lipid Metabolism in Plants* pp 563-584 ed. T S Moore (Boca Raton FL: CRC Press)

-------, Menassa R and Kleppinger-Sparace K F 1988 A preliminary analysis of fatty acid synthesis in pea roots; *Plant Physiol.* **87** 134-137

Stahl R J and Sparace S A 1991 Characterization of fatty acid biosynthesis in isolated pea root plastids; *Plant Physiol.* **96** 602-608

Stankovic Z S and Walker D A 1977 Photosynthesis by isolated chloroplasts: Some effects of adenylates and Pi; *Plant Physiol.* **59** 428-432

Stumpf P K 1980 Biosynthesis of saturated and unsaturated fatty acids; in *The Biochemistry of Plants*, Vol 4 pp 177-204 eds P K Stumpf and E E Conn (New York: Academic Press)

------- 1984 Fatty acid biosynthesis in higher plants; in *Fatty Acid Metabolism and Its Regulation* pp 155-179 ed. S Numa (New York: Elsevier)

------- 1987 Lipids: Structure and Function; in *The Biochemistry of Plants*, Vol 9 pp 121- 136 eds P K Stumpf and E E Conn (London: Academic Press Inc)

Tetlow I J, Blissett K J and Emes M J 1994 Starch synthesis and carbohydrate oxidation in amyloplasts from developing wheat endosperm; *Planta* **194** 454-460

-----, Bowsher C G and Emes M J 1996 Reconstitution of the hexose phosphate translocator from the envelope membranes of wheat endosperm amyloplasts; *Biochem. J.* **319** 717-723

------, Blissett K J and Emes M J 1998 Metabolic pools during starch synthesis and carbohydrate oxidation in amyloplasts isolated from wheat endosperm; *Planta* **204** 100-108

Thom E, Mohlmann T, Quick W P, Camara B and Neuhaus H E 1998 Sweet pepper plastids: enzymic equipment, characterization of the plastidic oxidative pentose phosphate pathway, and transport of phosphorylated intermediates across the envelope membrane; *Planta* **204** 226-233

Topfer R, Martini N and Schell J 1995 Modification of plant lipid synthesis; *Science* **268** 681-686

Trimming B A and Emes M J 1993 Glycolytic enzymes in non- photosynthetic plastids of pea (*Pisum sativum* L) roots; *Planta* **190** 439-445

Tyson R H and apRees T 1988 Starch synthesis by isolated amyloplasts from wheat endosperm; *Planta* **175** 33-38

Vick B and Beevers H 1978 Fatty acid synthesis in endosperm of young castor bean seedlings; *Plant Physiol.* **62** 173-178

Weaire P J and Kekwick R G O 1975 The synthesis of fatty acids in avocado mesocarp and cauliflower bud tissue; *Biochem. J.* **146** 425-431

Werdan K, Heldt H W, Ilovancev M M 1975 The role of *p*H in the regulation of carbon fixation in the chloroplast stroma: Studies on CO_2 fixation in the light and dark; *Biochim. Biophys. Acta* **396** 276-292

Williams M and Randall D D 1979 Pyruvate dehydrogenase complex from chloroplasts from Pisum sativum L; *Plant Physiol.* **64** 1099-1103

Xu D P, Sung S J S, Loboda T, Kormanik P P and Black C C 1989 Characterization of sucrolysis via the uridine diphosphate and PPi dependent sucrose synthase pathway; *Plant Physiol.* **90** 635-642

Yamada M and Nakamura Y 1975 Fatty acid synthesis by spinach chloroplasts. II. The path from PGA to fatty acids; *Plant Cell Physiol.* **16** 151-162

------- and Usami Q 1975 Long chain fatty acid synthesis in developing castor bean seeds . IV. The synthetic system in proplastids; *Plant Cell Physiol.* **16** 879-884