Chapter 11
Metabolic Engineering

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11.1 Introduction

Quality traits, including alterations in metabolite composition of crop plants, have been major targets of traditional breeding programs. One of the most prominent examples is the introduction of rape seed varieties low in erucid acid (in seed oil) and in glucosinolates (in meal) approximately 30 years ago which was an important step towards improving the nutritional properties of rape seed products. However, conventional breeding strategies depend on the availability of significant genetic variation for a given trait within the species gene pool and are further limited by the complex genetics underlying some quality traits. Moreover, due to its untargeted nature breeding of novel traits is time-consuming and slow. The emergence of molecular biology and plant transformation technologies offers the possibility of manipulating plant metabolism by a more rapid, targeted approach. The widespread adoption of transgenic plants in the past two decades gave rise to the discipline of plant metabolic engineering and provided enormous progress concerning the manipulation of plant metabolism. Basically, metabolic engineering was defined as the alteration of metabolic output by the introduction of recombinant DNA (Bailey 1991) or, more specifically, as the genetic modification of cellular biochemistry to introduce new properties or to modify existing ones (Jacobsen and Khosla 1998). The main goals of plant metabolic engineering are to produce valuable compounds in an economically attractive format, or to increase yield of a crop plant. On the level of metabolites these goals can be achieved by: (i) an increase in the production of a specific desired compound, (ii) the deletion or reduction of a specific unwanted product and (iii) the introduction of pathways leading to new products. In contrast to conventional breeding, transgenic strategies offer a rapid way to introduce

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desirable traits directly into the genome of elite varieties. It is clear, however, that the successful manipulation of plant metabolism requires detailed understanding of the underlying factors that regulate it. Traditionally, metabolic pathways have been analyzed on a step-by-step basis and limiting enzymes have been identified according to their biochemical properties or by using metabolic control analysis (ap Rees and Hill 1994; Geigenberger et al. 2004). Subsequently, enzyme overexpression is employed to alleviate metabolic bottlenecks. However, given the enormous flexibility of plant metabolism these direct approaches are often confounded by intervention of other limiting steps within the pathway, by counterbalancing regulation and by previously unrecognized competing pathways.

Despite these hurdles considerable success has been achieved in plant metabolic engineering also using single gene strategies. In this chapter, we briefly introduce the most common molecular strategies used to modulate plant metabolism, before detailing how these approaches have been used to engineer plant metabolism. Here, we focus on primary metabolism, namely carbohydrate and lipid metabolism. Finally, we give a short impression of a few successful examples of engineering secondary metabolism.

11.2 Strategies for Metabolic Engineering in Plants

Metabolic engineers have access to a vast array of molecular and genetic tools to rewire plant metabolism, most of which aims at the modulation of enzyme activity either toward an increase or a decrease of metabolic flux through a given pathway. In the simplest case a single enzymatic step is the target for modulation. To increase the production of a desired compound or a novel compound, genes encoding biosynthetic enzymes of the pathway can be overexpressed. Further increases in flux can be achieved by overexpressing enzymes from heterologous sources which are not subject to regulation or which have different regulatory properties compared to the endogenous plant enzyme. A problem associated with overexpression of single enzymes is that other steps in the pathway can become limiting and thus total metabolic flux does not substantially increase. To circumvent this, several consecutive enzymes in the same pathway must be up-regulated at the same time, either by transferring several expression cassettes into the plant or by overexpression of regulatory proteins, i.e. transcription factors. The latter approach, however, requires transcriptional co-regulation of all steps in a pathway as it has been shown for a number of pathways in plant secondary metabolism (Broun 2004; Grotewold 2008).

To reduce the levels of undesirable gene products, two general approaches are commonly used: recessive gene disruption and dominant gene silencing. In gene disruption approaches, the target sequence is mutated to eliminate a particular gene function, whereas dominant gene silencing methods induce either the destruction of the gene transcript or the inhibition of transcription. So far, directed gene disruption is not efficient in higher plants. Therefore, the most widely used

technologies for the generation of loss-of-function mutants are transposon mutagenesis (Altmann et al. 1995; Kim et al. 2004), *Agrobacterium* T-DNA insertions (An et al. 2003; Jeon et al. 2000; see also Chap. 1) and, more recently, the use of chemical mutagenesis in combination with TILLING (Henikoff et al. 2004) to create disruptions in coding regions of genes. These techniques have been proven very useful for functional genomics; however, their use for metabolic engineering is limited. They are restricted to a few genetically tractable plant species and due to their untargeted nature they require the generation of large populations of mutated plants to screen for a desired mutation. In addition, genetic redundancy caused by multi-gene families and polyploidy further complicates these kinds of knock-out approaches.

RNA interference (RNAi) and related mechanisms such as 'antisense' or 'co-suppression' are homology-dependent gene-silencing technologies that possess a great potential for metabolic engineering (Mansoor et al. 2006; Tang et al. 2007; see Chap. 5 for details on the mechanism). In comparison with gene disruption, RNA silencing bears several advantages. It is a dominant trait that can be introduced into any transformable plant species, including the transfer into elite crop varieties. Owing to its targeted nature it does not require the generation of overly large populations of transgenic plants to find a suitable event. Especially in the case of antisense or co-suppression, the efficiency of silencing can vary considerably between individual transformants. This allows the manipulation of metabolic steps where a loss-of-function would be detrimental to the plant but a decrease of gene expression at 30–90% yields a desired metabolic phenotype. Furthermore, by the use of specific promoters, RNA silencing can be manipulated in a spatial and temporal manner. This is important for genes where down-regulation is good for the improvement of a specific organ, e.g. seeds or tubers, but is deleterious to the growth of other plant organs.

Examples are provided below to show how the above strategies are applied to manipulate the production of different classes of compounds.

11.3 Engineering of Primary Metabolism

11.3.1 Carbohydrate Metabolism

The majority of metabolic fluxes inside a plant cell center on the formation and utilization of sugars, the primary products of photosynthesis and their conversion into storage and structural carbohydrates, such as starch and cellulose. Starch is the principle constituent of many of harvestable organs, such as tubers or grain. Besides its importance as a staple in human and animal diets, it is also used as a renewable raw material for a wide range of industrial applications (Jobling 2004). Starch is a relatively simple polymer composed of glucose molecules that are linked in two different forms. Amylose is an essentially linear polymer in which the glucose

moieties are joined end-to-end by α(1→4) linkages. Amylopectin is a much larger branched molecule, in which about 5% of the glucose units are joined by α(1→6) linkages. The ratio between amylose and amylopectin is dependent on the plant species or variety, respectively, and is one determinant of the physico-chemical properties of plant derived starches which are important for technical uses. The biochemical pathways leading to starch formation are well documented and the key enzymatic steps have been identified (Fernie et al. 2002; Geigenberger 2003). Starch metabolism in potato tubers is particularly well characterized and attempts to both increase the accumulation of starch and to modify its structural properties by metabolic engineering have received considerable attention (see also Chap. 20). For starch synthesis in growing potato tubers, sucrose delivered from the phloem is cleaved by sucrose-synthase into uridine-diphosphoryl-glucose (UDP-glucose) and fructose, which are converted to hexose phosphates by UDP-glucose pyrophosphorylase and fructokinase, respectively. Glucose-6-phosphate is then imported into the amyloplast via a glucose-6-phosphate transporter (GPT; Kammerer et al. 1998) and is converted via plastidial phosphoglucomutase and ADP-glucose pyrophosphorylase (AGPase) to ADP-glucose (Fig. 11.1). This process requires

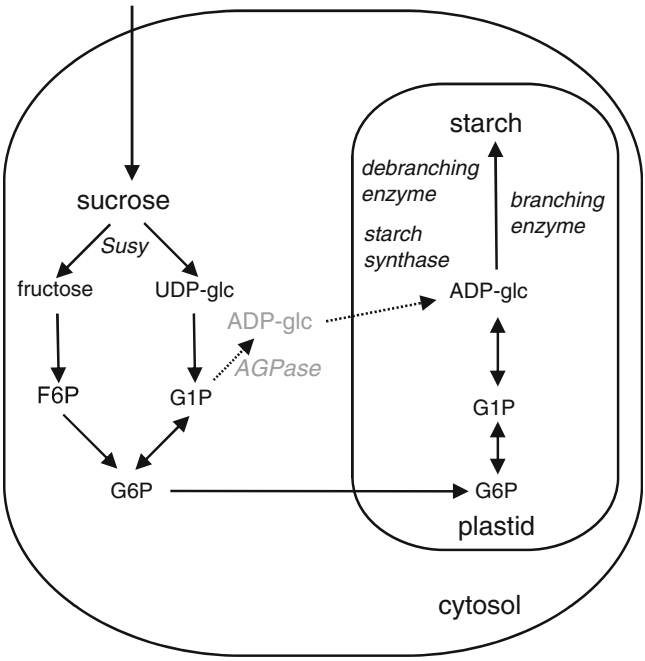

Fig. 11.1 Principal pathway leading to the formation of starch in storage organs. The alternative route of ADP-glc via generation within the cytosol and subsequent uptake into the amyloplast, as it occurs in the endosperm cells of graminaceous species, is shown by *dotted arrows*. *Susy* sucrose synthase, *UDP-glc* UDP-glucose, *F6P* fructose-6-phosphate, *G1P* glucose-6-phosphate, *AGPase* ADP-glucose pyrophosphorylase, *ADP-glc* ADP-glucose

ATP, which is imported into the amyloplast via an ATP transporter (Tjaden et al. 1998). The glycosyl moiety of ADP-glucose is the substrate for the synthesis of starch via various isoforms of starch synthase.

Starch synthesis in the endosperm of cereals differs from that in other organs in that the synthesis of ADP-glucose occurs in the cytosol, via a cytoplasmic isoform of AGPase. ADP-glucose is imported into the plastid via a specific nucleotide transporter (Tomlinson and Denyer 2003).

To increase the efficiency of the pathway and thus to increase starch accumulation in crop plants, molecular strategies have initially concentrated on AGPase, the enzyme assumed to catalyze the rate-limiting step of starch synthesis. In an early attempt to increase the activity of the starch biosynthetic pathway in potato tubers, Stark et al. (1992) overexpressed a deregulated bacterial AGPase in the potato variety Russet Burbank. Overall, the transformed lines were reported to have an average of 35% more tuber starch than the controls. However, this effect was lost upon transformation of a different potato cultivar (Sweetlove et al. 1996). In the latter case, starch degradation was up-regulated in addition to starch synthesis, resulting in no net change in starch accumulation.

Attempts to increase starch contents through manipulation of AGPase in cereal seeds have made use of a variant of the maize AGPase gene (*shrunken2*) whose gene product is less sensitive to inhibition by phosphate when compared to the wild-type protein.

Smidansky and colleagues (2002, 2003, 2007) showed that maize, rice and wheat plants expressing this AGPase allele in the endosperm and grown under controlled conditions display an increase in individual seed weight as well as in seed yield per plant. However, in field trials transgenic wheat plants only showed a yield enhancement under conditions of minimal inter-plant competition and optimal water supply (Meyer et al. 2007).

Starch content in potato tubers is very sensitive to manipulation of the plastidial adenylate transporter providing the ATP necessary for the AGPase reaction. Overexpression of an adenylate transporter from *Arabidopsis* in potato tubers resulted in 16–36% more starch per gram fresh weight, indicating that ATP supply to the plastid limits starch synthesis (Tjaden et al. 1998; Geigenberger et al. 2001). Recently, a further increase in potato tuber starch content was achieved by the simultaneous overexpression of a GPT from pea and an *Arabidopsis* adenylate translocator. Double transformants exhibited an increase in tuber yield of up to 19% in addition to an increase in starch content of 28%, when compared to control plants (Zhang et al. 2008). Both effects taken together led to a calculated increase in potato tuber starch of up to 44%. The authors concluded that starch synthesis in potato tubers is co-limited by the ATP supply as well as by the import of carbon skeletons into the amyloplast (Zhang et al. 2008). Further evidence for an energy limitation of starch synthesis in potato tubers comes from transgenic plants with reduced expression of plastidial adenylate kinase (ADK; Regierer et al. 2002). In this study a strong negative influence of ADK activity on starch accumulation was found, suggesting that ADK normally competes with starch synthesis for plastidial ATP.

Taken together, successful attempts to increase starch content through metabolic engineering are scarce. The analyses so far suggest that in potato tubers considerable control of starch synthesis lies outside of the linear pathway as both the adenylate transporter as well as the plastidial ADK appear to exert higher control over the pathway than AGPase, the enzyme widely believed to be rate-limiting (Geigenberger et al. 2004).

To provide improved raw material for the starch processing industry considerable effort has been aimed at altering starch quality which is mainly defined by the amylopectin to amylose ratio (Jobling 2004). Most of the work in this direction has been done on potato tubers as these are one of the major sources for industrial starches.

The synthesis of amylose is accomplished through the activity of a particular isoform of starch synthase, GBSS, and antisense inhibition of this gene leads to amylose-free potato starch (Visser et al. 1991). Amylose-free potato starch can be expected to find application in both the food industry and in paper manufacture. Large-scale field trials with transgenic amylose-free potato varieties have been conducted in Europe and this crop is currently going through the regulatory approval process.

High-amylose starches are also of great interest, e.g. for improved frying or for industrial use as gelling agents or thickeners. Recently, an innovative approach to increase the amylose content in potato tubers involved the inhibition of starch-branching enzyme A (SBE A) activity, the enzyme responsible for the introduction of $\alpha 1 \rightarrow 6$ linkages into amylopectin (Jobling et al. 2003). The authors of this study expressed a single-chain antibody targeted against the active site of SBE A in transgenic potato tubers, thereby neutralizing the enzymatic activity. They found that immunomodulation of SBE A increased the amylose content of starch granules from about 20% in wild-type tubers to 74% in the best transgenic line, exceeding the concentration of amylose achieved be conventional antisense strategies (Jobling et al. 2003).

11.3.1.1 Production of Novel Carbohydrates in Transgenic Plants

In addition to attempts aiming at manipulating the contents and properties of endogenous carbohydrates, there have been several successful approaches for the production of novel carbohydrates in transgenic plants.

Fructans, or polyfructosylsucroses, are an alternative storage carbohydrate that are highly soluble and are stored within the vacuole. Fructans are present in approximately 15% of all flowering plants (Hellwege et al. 2000). Fructan synthesis is initiated by sucrose:sucrose 1-fructosyltransferase (SST) which catalyzes the fructosyl transfer from one sucrose molecule to another, resulting in the trisaccharide 1-kestose. In subsequent steps, fructosyltransferase (FFT) catalyzes the reversible transfer of fructosyl residues from one fructan to another, producing a mixture

of fructans with different chain length (Ritsema and Smeekens 2003). One of the simplest fructans is inulin, which consists of β(1→2)-linked fructose residues while fructans of the levan type are β(2→6)-linked fructose polymers.

From a biotechnological viewpoint, interest in fructans has continued to increase, as they have been recognized as beneficial food ingredients. As part of the human diet, they are considered to be prebiotics as they selectively promote the growth of beneficial intestinal bacteria. Furthermore, fructans are assumed to have anti-cancer activity, promote mineral absorption, decrease cholesterol levels and decrease insulin levels. Fructans are normally isolated from plants with low agronomic value, such as the Jerusalem artichoke (*Helianthus tuberosus*) and chicory (vegetables are also covered in Chap. 25). Thus, attempts have been made to produce transgenic plants with higher fructan yield or making fructans with specific properties. Transformation of sugar beet with an SST gene from Jerusalem artichoke resulted in the conversion of 90% of the vacuolar sucrose into fructan (Sevenier et al. 1998), since the sugar beet accumulates to concentrations approaching 600 mM sucrose, this represents a massive fructan yield. Other researchers have introduced an SST along with an FFT from onion into sugar beet which resulted in an efficient conversion of sucrose into complex, onion-type fructans, without the loss of storage carbohydrate content (Weyens et al. 2004). Potato, as another crop naturally not accumulating fructans, was used to express plant fructosyltransferases. The SST and FFT enzymes from globe artichoke were engineered into potato and led to the accumulation of the full range of fructans found in globe artichoke itself (Hellwege et al. 2000).

Another recent example of the use of potato tubers as bioreactors is the production of isomaltulose (IM), a sucrose isomer that is an excellent sucrose substitute in foods as it shares many physico-chemical properties with sucrose but is non-metabolizable and non-cariogenic. A gene encoding a sucrose isomerase (*palI*) which catalyzes the conversion of sucrose into IM has been isolated from the bacterium *Erwinia rhapontici* (Börnke et al. 2001). Expression of the *palI* gene within the apoplast of transgenic potato tubers led to a nearly quantitative conversion of sucrose into IM. Despite the soluble carbohydrates having been altered within the tubers, growth of PalI expressing transgenic potato plants was indistinguishable from wild-type plants. Therefore, expression of a bacterial sucrose isomerase provides a valid tool for high level IM production in storage tissues of transgenic crop plants (Börnke et al. 2002). Towards this direction, Wu and Birch (2007) introduced a sucrose isomerase gene tailored for vacuolar compartmentation into sugar cane. Transgenic lines accumulated substantial amounts of IM in their culm. Remarkably, this was not at the expense of sucrose levels, resulting in up to doubled total sugar concentration in juice harvested from sucrose isomerase expressing transgenic sugar cane lines. The reason for this boost in sugar concentration is not understood but it has been hypothesized that IM accumulation in the culm leads to enhanced sink strength that fosters import of additional carbon from source tissues (Wu and Birch 2007). It remains to be shown whether this strategy allows increasing total sugar content in other sucrose storing crops such as sugar beet.

11.3.2 Metabolic Engineering of Lipid Metabolism

A plant cell contains a plethora of lipid species, which are mainly represented by free fatty acids, glycolipids, phospholipids, waxes and neutral glycerolipids. Vegetable oil for human consumption almost exclusively consists of triacylglycerols (TAGs), which are composed of three fatty acids esterified to glycerol. TAGs dominate the storage lipid pool in oilseeds from which most plant oils are isolated. There are five fatty acids that are commonly esterified to triglycerides in the predominant oilseed crops (Dyer et al. 2008; see also Chap. 21), namely palmitic acid (16:0), stearic acid (18:0), oleic acid ($18:1\Delta^{9}$), linoleic acid ($18:2\Delta^{9,12}$) and α-linolenic acid ($18:3\Delta^{9,12,15}$). In order to obtain plant oil with improved technological or nutritional value by metabolic engineering, either unusual fatty acids that are highly abundant in exotic non-crops or novel fatty acids are produced in oilseeds, which can be cultivated on a large scale in contrast to the species from which these unusual fatty acids originate. As a prerequisite for efficient retrieval of the engineered fatty acids, these need to be targeted into storage lipids of seed oil.

Before we discuss potential applications of plants producing unusual fatty acids and the limitations and bottlenecks that were encountered upon engineering of glycerolipids in transgenic oilseed crops, we will briefly outline the route of triacylglycerol biosynthesis.

11.3.2.1 Biosynthesis of Storage Lipids

The biosynthesis of glycerolipids (triacylglycerols; TAGs) is a complex, non-linear pathway that involves three subcellular compartments, the chloroplast, the cytosol and the endoplasmatic reticulum (ER). Utilizing the photochemically generated reducing power in the chloroplast stroma, the *de novo* biosynthesis of palmitic acid (16:0), stearic acid (18:0) and oleic acid ($18:1\Delta^{9}$) from activated Acetyl-CoA building blocks takes place while the nascent acyl chain is covalently bound to acyl carrier protein (ACP) complex. Acyl-ACP can undergo two different fates:

1. For the biosynthesis of phospholipids and galactolipids at the chloroplast envelope, the acyl chains can be directly transferred from acyl-ACPs to glycerol-3-phosphate and subsequently to lysophosphatidic acid (LPA). The product, phosphatidic acid (PA), represents the substrate for the production of phospholipids via the transfer of choline, ethanolamine or serine. However, phospholipid synthesis via the prokaryotic plastidic pathway has a very minor impact on seed oil production and is not discussed further.
2. More importantly, fatty acids can be liberated from the plastidic acyl-ACP pool and transferred to the cytosol (Fig. 11.2), where they are: (i) re-esterified to coenzyme A and (ii) subsequently incorporated into the phospholipid pool at the ER. While acyl-CoAs are the substrates for elongases at the cytosolic leaflet of the ER, the cytochrome b_5 containing desaturases FAD2 and FAD3 utilize the phospholipid-bound fatty acid pool (Ohlrogge and Browse 1995).

Precursors and intermediates for TAG biosynthesis at the ER derive from both free acyl-CoA thioesters and phospholipids. The route of TAG synthesis via glycerol-3-P and free acyl-CoA is known as the Kennedy pathway (Fig. 11.2) and involves glycerol-3-phosphate acyltransferase (GPAT), lysophosphatidic acid acyltransferase (LPAAT), phosphatidic acid phosphatase (PAP) and diacylglycerol acyltransferase (DGAT). In each of the three acyl transferase steps (GPAT, LPAAT, DGAT), one more acyl chain is esterified to the glycerol backbone. Alternatively (see Fig. 11.2), fatty acids can be directly transferred from the phospholipid pool into TAG by phospholipid:diacylglycerol acyltransferase (PDAT) or enter the diacylglycerol (DAG) pool by the reversible removal of the phospholipid head group via choline phosphotransferase (CPT). DAG can then be utilized by DGAT or PDAT to yield TAG.

In the production of novel plant oils, both DGAT (Jako et al. 2001; Yu et al. 2006) and PDAT (Dahlqvist et al. 2000) were found to represent rate limiting steps for the entry of heterologously produced fatty acids into the TAG pool in metabolically engineered oilseeds (Bates et al. 2007), identifying the ultimate step

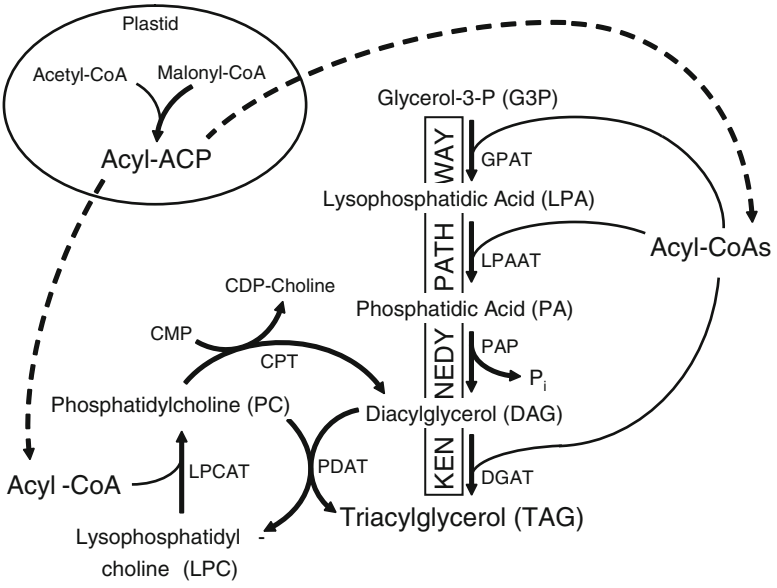

Fig. 11.2 Routes for triacylglycerol biosynthesis in oilseeds. Triacylglycerols (TAGs) can be synthesized from the glycerol-3-phosphate and the acyl-CoA pool via the Kennedy pathway by subsequent acylation of the triose backbone. Alternatively, the penultimate intermediate, diacylglycerol (*DAG*) and the TAG end-product can be generated via acyl transfer from the phospholipid pool. Please see the text for more detailed explanation (modified after Napier 2007 and Dyer et al. 2008). *Acetyl-CoA* Acetyl coenzyme A, *Acyl-ACP* acylated acyl carrier protein, *CPT* choline phosphotransferase, *DGAT* diacylglycerol acyltransferase, *GPAT* glycerol-3-phosphate acyltransferase, *LPAAT* lysophosphatidic acid acyltransferase, *LPCAT* lysophosphatidylcholine acyltransferase, *Malonyl-CoA* malonyl coenzyme A, *PAP* phosphatidic acid phosphatase, *PDAT* phospholipids:diacylglycerol acyltransferase

of TAG biosynthesis as a committed entry site for fatty acids. However additional bottlenecks for the flux of novel fatty acids into TAG were identified, which are discussed later.

11.3.2.2 Genetic Engineering of Plant Lipid Metabolism

The manipulation of lipid metabolism in genetically engineered plants provides an enormous economic potential. The world annual production of vegetable oils amounts to 128.2×10^6 t in 2007, which is only 30× lower than the annual production of crude mineral oil of 4100×10^6 t/year. In contrast to mineral oil, plant oils represent both a renewable resource and a versatile commodity for food, feed and industrial applications. About 14% of the annual plant oil production is being used for industrial processing, 5% are used as feed and for biodiesel production, respectively, while the rest is consumed as human food (Metzger and Bornscheuer 2006; Durrett et al. 2008).

Soybean, oil palm, rapeseed and sunflower are the predominant oil crops in the world (Dyer et al. 2008; also covered in Chaps. 21, 24). Other important oil crops are peanut, cottonseed, palm kernel, coconut and olives. However, more than 60% of the annual vegetable oil production is derived from soybean and palm oil. As summarized by Dyer et al. (2008), seed oil from these major oilseeds is mainly composed of the five major fatty acids palmitic acid (16:0), stearic acid (18:0), oleic acid ($18{:}1\Delta^9$), linoleic acid ($18{:}2\Delta^{9,12}$) and α-linolenic acid ($18{:}3\Delta^{9,12,15}$). Vegetable oil enriched for fatty acids uncommon to these major oilseed crops and fatty acids with additional functions provide a huge potential as chemical feedstock for the industrial production of detergents, cosmetics, drying oil, paint, ink, specialized lubricants or plastics providing a much higher versatility than mineral oil (Metzger and Bornscheuer 2006; Dyer et al. 2008). Consequently, engineering the lipid composition of seed oil has mainly followed three objectives: (i) to produce unusual fatty acids in oil crops that are of special value as chemical feedstock, (ii) to generate a fatty acid composition optimized for chemical processing and (iii) to introduce fatty acids with a special nutritional value like very long polyunsaturated fatty acids (VL-PUFAs).

In the following, we discuss the current advance in the production of: (i) unusual, short chain fatty acids like lauric acid (12:0), caprylic acid (8:0) and capric acid (10:0), (ii) long-chain fatty acids like erucic acid (22:1) and very-long-chain polyunsaturated fatty acids (VL-PUFAs) like arachidonic acid (AA; 22:4), eicosapentanoic acid (EPA; 20:5) and docosahexaenoic acid (DHA; 22:6) as well as (iii) various fatty acids with additional functional groups in transgenic oilseed crops.

Unusual Medium-Chain Fatty Acids

Glycerolipids (TAGs) containing medium-chain acyl residues are of outstanding interest for the use as biofuel. Medium-chain TAGs are devoid of two

major disadvantages intrinsic to conventional biodiesel consisting of TAG containing the five major fatty acids (Durrett et al. 2008). First, complications caused by biodiesel viscosity are alleviated when medium-chain TAGs are used, as TAG viscosity decreases with the chain length of the esterified fatty acids. The viscosity of regular biodiesel is tenfold higher compared to fossil fuel and is commonly prevented by utilizing fatty acid methyl esters (FAMEs) after the trans-esterification of the TAG fatty acids to methanol. Second, the coking index of medium-chain containing TAGs is lower, compared to other fuels.

The most distinguished example for metabolic engineering of medium-chain fatty acids in oilseeds is the generation of transgenic high-lauric acid (12:0) rapeseed, which is currently approved for commercial use. In the initial approaches, the overexpression of a laureate-specific ACP from the California bay tree (*Umbellularia californica*) in *Arabidopsis* and *Brassica napus* (rapeseed) led to an accumulation of more than 50% of lauric acid in seed TAGs (Voelker et al. 1992; Wiberg et al. 2000). However, the *sn*-2 position of glycerol barely contained lauroyl residues in these transgenics. Additional overexpression of a LPAAT from coconut with high specificity for lauroyl-CoA increased the yield of laureate in rapeseed TAG to 67%, indicating that a limitation in the Kennedy pathway (see Fig. 11.2) restricted the accumulation of lauric acid in seed oil of the transgenics (Knutzon et al. 1999). Likewise, *Cuphea lanceolata*, which accumulates more than 80% of capric acid (10:0) in seed TAG was found to contain one set of GPAT and LPAAT specific for medium-chain acyl-CoAs and an DGAT that preferentially funnels di-medium-chain DAGs into TAG (Dehesh 2001). The specificities of these three Kennedy pathway enzymes obviously leads to an effective channelling of medium-chain fatty acids into *Cuphea* TAG.

Attempts to introduce valuable medium-chain fatty acids like capric acid (10:0) or caprylic acid (8:0) into rapeseed TAG were less successful, leading to 8% and 30% medium-chain acyls residues in rapeseed oil (Wiberg et al. 2000). However, the acyl-CoA pool in the transgenic seeds was dominated by the introduced medium-chain fatty acids, again indicating that the incorporation into glycerolipids via the Kennedy pathway was the limiting step preventing a high yield of caprylic and capric acid in the TAG pool (Larson et al. 2002).

Unusual Long-Chain Fatty Acids

Coriander and *Thunbergia alata* seed oil contain more than 80% of the unsusual monoenoic fatty acids petroselinic acid ($18{:}1\Delta^6$) and $16{:}1\Delta^6$, respectively, both of which are valuable precursors for the production of various plastic polymers and cyclic hydrocarbon skeletons. Interestingly, both unusual fatty acids are synthesized by plastidic acyl-ACP desaturases. Palmitoyl-ACP (16:0-ACP) is utilized as a substrate for desaturation at the Δ^4 and Δ^6 position, respectively and the monoenoic fatty acid products are targeted to seed TAG via the phospholipid pool at the ER (Cahoon and Ohlrogge 1994; Schultz et al. 2000).

The 16:1Δ^4 product of the coriander desaturase is then subsequently elongated to yield petroselinic acid, while the 16:1Δ^6 fatty acid is a direct product of the *Thunbergia* desaturase. The accumulation of these two unusual monoenoic fatty acids in transgenic *Arabidopsis* overexpressing the coriander and *Thunbergia* ACP-desaturases amounted to less than 15% of total seed TAG (Suh et al. 2002). In coriander, specific ACP, 3-ketoacyl-ACP synthase and thiesterase are present for the synthesis of petroselinic acid in plastids (Suh et al. 2002), suggesting that an inefficient substrate channelling between the prokaryotic pathway enzymes in *Arabidopsis* and the heterologously expressed desaturase may be the cause to the relatively low abundance of petroselinic acid in seed oil of the transgenics.

In contrast to petroselinic acid, erucic acid (22:1 Δ^{13}) is produced in high amount in oilseed rape and other Brassicaceae. However, erucic acid is largely restricted to the *sn*-1 and *sn*-3 positions of TAG. Again, the specificity of the endogenous LPAAT seems to prevent the incorporation of erucic acid at the *sn*-2 position, identifying the same bottleneck that limited lauric acid accumulation in TAG of transgenic rapeseed. When an LPAAT from *Limanthes* specific for erucic acid and the endogenous FAE1 elongase were overexpressed in parallel, the TAG pool of the resulting transgenic rapeseed contained more than 70% erucic acid (Nath et al. 2006).

The production of the very-long-chain polyunsaturated fatty acids (VL-PUFAs) AA (an ω6-fatty acid), EPA (an ω3-fatty acid) and DHA (an ω3-fatty acid) has drawn considerable attention due to their importance for human nutrition. Furthermore, the application of VL-PUFAs isolated from transgenic oilseed crops as a feed supplement to enable more sustainable salmon farming was supposed (Cahoon et al. 2007). Nevertheless, the production of VL-PUFAs in transgenic plants is complicated as it involves several cycles of desaturation and chain elongation of the endogenous precursors linoleic acid (18:2) and α-linolenic acid (18:3). As outlined in the previous section, the substrates for fatty acid desaturases are PC bound fatty acids, while elongases use free acyl-CoAs as their substrates, necessitating a substrate shuttling between the phospholipid and the acyl-CoA pool. Metabolic engineering of transgenic plants for VL-PUFA production has been accomplished by the introduction of several desaturases and elongases in *Arabidopsis* and *Brassica juncea*, totalling to up to nine transgenes (Wu et al. 2005). However, various routes can be chosen for VL-PUFA production. Apart from the Δ^6 desaturase pathway, on which most attention has been focused to date, as it allows for the simultaneous biosynthesis of AA, EPA and DHA, the Δ^8 desaturase pathway has proven an interesting alternative for the production of AA and EPA (Qi et al. 2004) Commonly, the maximum yield of VL-PUFAs in TAG of transgenic *Arabidopsis*, *Brassica juncea* and soybean obtained to date is low and ranges between 3% for DHA (Wu et al. 2005; Kinney 2006) and 8% for EPA (Qi et al. 2004; Wu et al. 2005). Recently, desaturases that act on acyl-CoAs have been identified from microalgae and higher plants, possibly making trans-esterification between acyl lipids and the acyl CoA pool dispensable in the

future, which could also improve the yield of VL-PUFAs (Sayanova et al. 2007; Hoffmann et al. 2008).

Fatty Acids with Additional Functional Groups

Fatty acids with additional functional groups and their chemical derivatives represent an emerging valuable resource as industrial feedstocks for the production of cometics, lubricants, nylon, resins, polyvinylchloride (PVC), polyurethane and drying oils in paint and ink (Metzger and Bornscheuer 2006). Here, we briefly discuss unusual fatty acids that contain hydroxyl, epoxy and stereochemically unusually conjugated hexatriene groups, which have in common that they all are synthesized by divergent forms of the ER Δ^{12}-oleic acid desaturase FAD2 (van de Loo et al. 1995; Lee et al. 1998; Dyer et al. 2002).

Ricinoleic acid is produced by a Δ^{12}-hydroxylase and represents almost 90% of the castor bean (*Ricinus communis*) seed oil pool. Ricinoleic acid carries a hydroxyl group at the C-12 position in addition to a cis-double bond at the C-9 position, which renders it to a versatile substrate for various organic syntheses (Metzger and Bornscheuer 2006). Vernolic acid is synthesized by a Δ^{12}-epoxygenase and is abundant in the seed oil of, e.g. *Vernonia galamensis*, *Crepis palaestina* and *Euphorbia lagascae*. It contains an epoxy group at position C-12 in addition to the C-9 double bond and can be used as a binder in coatings and for the synthesis of enantiomerically pure products. Calendulic acid ($18{:}3\Delta^{8trans,10trans,12cis}$) and α-eleostearic acid ($18{:}3\Delta^{9cis,11trans,13trans}$), which are abundant in the seed TAG pool of marigold (*Calendula officinalis*) and the Chinese tung tree (*Vernicia fordii*), respectively, are used as drying oils in paints, inks and coatings. The conjugated hexatrienic double bonds of calendulic and α-eleostearic acid are synthesized from linoleic acid by a FAD2 conjugase (Cahoon et al. 1999).

Intriguingly, the overexpression of these three divergent FAD2 genes in Arabidopsis and soybean lead to less than 20% accumulation of ricinoleic, vernolic, calendulic and α-eleostearic acid in seed TAG as compared to 60% to 90% in the native species (Broun and Somerville 1997; Lee et al. 1998; Cahoon et al. 1999). Instead, oleic acids contents were increased in all these transgenics and the unusual fatty acids accumulated in the PC pool (Thomaeus et al. 2001; Cahoon et al. 2006), indicating that: (i) the conversion from oleic to linoleic acid by the endogenouse FAD2 desaturase is disturbed by the transgene expression and (ii) the channelling of the unusual fatty acids into the TAG pool is inefficient in the transgenics. In *Vernonia galamensis*, castor bean and tung tree, the respective DGAT2 isoforms were identified to specifically confer the transfer of vernolic, ricinoleic and α-eleostearic acid into seed oil, respectively (Cahoon et al. 2006; Kroon et al. 2006; Shockey et al. 2006), identifying DGAT as the potential bottleneck for the accumulation of these fatty acids in the TAG pool.

11.4 Engineering of Secondary Metabolism for Human Health and Nutrition

Plants produce a large array of secondary metabolites. These are loosely defined as organic compounds with no essential role in growth and development. Although not absolutely required, these compounds confer some selective advantage for the plant and many have been implicated in the plants' interaction with its immediate environment. Plant secondary compounds are commonly consumed as part of the human diet and they play an important role as phytonutrients as they are assumed to offer protection against certain cancers, cardio-vascular diseases, act as antioxidants or bear other health promoting properties. Due to their presumed health benefits, there is growing interest in the development of food crops with tailor-made levels and composition of secondary compounds, designed to exert an optimal biological effect.

Given the wealth of plant secondary compounds relevant for human nutrition, we concentrate here on a few recent examples which highlight the potential of engineering plant secondary metabolism. For a more comprehensive overview, the reader is referred to some excellent recent reviews (e.g. Kinney 2006; Zhu et al. 2007).

11.4.1 Flavonoids

Flavonoids are phenolic compounds derived ultimately from phenylalanine which impart much of the color and flavor of fruits, vegetables, nuts and seeds. The first committed step in flavonoid biosynthesis is the conversion of the precursor 4-coumaroyl-CoA into chalcone by the enzyme chalcone synthase. Chalcone is then derivatized in a series of enzymatic steps to eventually form different classes of flavonoids, such as flavanones, dihydroflavonols and finally to the anthocyanins, the major water-soluble pigments in flowers and fruits (Schijlen et al. 2004). Tomato is an excellent candidate for transgenic enhancement of flavonoid content. It is an important food crop worldwide; however, its flavonoid content is generally low and largely confined to the tomato peel. Constitutive, high level overexpression of a petunia chalcone isomerase in tomato resulted in up to 78-fold increases in the levels flavonoids in the peel (Muir et al. 2001). However, since the peel accounts for only about 5% of fruit mass the overall increase was rather low. A 3.5-fold increase in fruit flavonol content of tomato was achieved by RNAi-mediated suppression of the tomato *DET1* gene, which encodes a transcription factor negatively regulating photomorphogenic responses (Davuluri et al. 2005).

Coordinate transcriptional control of biosynthetic genes has emerged as a major mechanism dictating the final levels of secondary metabolites in plant cells. In various plant species the tissue-specific regulation of the structural genes involved in flavonoid biosynthesis is controlled by the combination of regulators

from two transcription factor families (Schijlen et al. 2004). Consequently, overexpression of *Lc* and *C1*, two transcription factors that control flavonoid biosynthesis in maize, resulted in tomato fruit containing 20-fold higher flavonol content than the respective control. In a similar approach, Butelli et al. (2008) expressed the *Del* and *Ros1* genes from snapdragon in the fruit of transgenic tomatoes. Both genes encode transcription factors that interact with each other to induce anthocyanin biosynthesis in snapdragon flowers. The fruit of the transgenic tomato plants accumulated anthocyanins at levels substantially higher than previously reported. Evidence for a health promoting effect of these engineered tomato fruits comes from a pilot study in which a cancer-susceptible mouse strain showed a significant extension of life span when fed on high-anthocyanin tomatoes (Butelli et al. 2008).

11.4.2 Vitamins

Vitamins are a chemically diverse group of organic compounds which are are classified by their biological and chemical activity, not their structure. Humans have to acquire a number of vitamins with their diet and many of these compounds are plant derived products. Vitamin deficiency is a serious problem in the developing world and optimizing vitamin content of plants through genetic engineering has received much attention in recent years (Herbers 2003; Zhu et al. 2007). Thus far, metabolic engineering has resulted in transgenic plants that contain elevated levels of provitamin A, vitamin C, E and folate (Ye et al. 2000; Agius et al. 2003; Cahoon et al. 2003; Storozhenko et al. 2007).

The principal example of vitamin metabolic engineering in plants is the synthesis of β-carotene (provitamin A, a carotenoid) in rice endosperm, which led to the development of so-called 'golden rice' varieties (Ye et al. 2000; Al-Babili and Beyer 2005). Carotenoids do not accumulate in rice endosperm; however, the precursor geranylgeranyl pyrophosphate is abundant. By introducing heterologous activities of a phytoene synthase, a phytoene desaturase, carotene desaturase (the latter two activities were mediated by a single bacterial enzyme) and lycopene β-cyclase, Ye et al. (2000) were able to produce rice with up to 2 μg/g β-carotene dry weight. Additional golden rice varieties have been generated that contain only two recombinant enzymes (daffodil phytoene synthase and *Erwinia* phytoene desaturase; Beyer et al. 2002); and most recently, a novel variety has been developed in which the daffodil phytoene synthase has been replaced by the more efficient maize homolog, resulting in a 23-fold improvement in β-carotene content (Paine et al. 2005).

Whereas vitamin A is a single compound, eight tocochromanols belong to the vitamin E family that differ in their methylation pattern of the polar head group and the saturation of the prenyl tail of the amphiphilic antioxidant. Due to the specificity of the retrieval system, α-tocopherol has the highest vitamin E activity in humans. The pathway of tocochromanol biosynthesis in plants has been characterized and the involved genes have been cloned (DellaPenna and Last 2006; DellaPenna and Pogson 2006). Work on transgenic plants has shown that the levels of vitamin E

activity can be increased either by increasing the total amount of tocochromanols or by shifting the metabolic flux towards α-tocopherol. For example, expression of a *Synechocystis* PCC6803 or *Arabidopsis* γ-tocopherol methyltransferase in *Arabidopsis* seeds resulted in a fundamental shift from γ/δ- to α/β-tocopherol without altering total vitamin E levels (Shintani and DellaPenna 1998). Similarily, transgenic soybean with more than 95% α-tocopherol in seeds that usually only contain 10% α-tocopherol were generated by the simultaneous overexpression of two methyltransferases from the *Arabidopsis* tocopherol biosynthetic pathway, AtVTE3 and AtVTE4 (Van Eenennaam et al. 2003). A 10- to 15-fold increase in total vitamin E has been achieved in *Arabidopsis* leaves by overexpression of a homogentisic acid geranylgeranyl transferase (HGGT) from barley while expression of the same activity in corn seeds increased vitamin E content by a factor of six (Cahoon et al. 2003). An 8-fold increase in total leaf tocochromanol content was obtained when yeast prephenate dehydratase (PDH) and Arabidopsis hydroxyphenylpyruvate dioxygenase (HPPD) were overexpressed in tobacco, thereby circumventing substrate limitation in the endogenous pathway (Rippert et al., 2004).

11.5 Conclusions

Recent years have seen a vast improvement of our understanding of plant metabolism at the genetic level and the interaction of metabolic pathways at the physiological level. The examples described above illustrate how this knowledge enables successful manipulation of metabolic networks via the overexpression or repression of single genes; however, these success stories are yet relatively rare. Multi-point metabolic engineering is now beginning to supersede single-gene manipulation as the most promising way to manipulate metabolic fluxes. The analytical tools available in the post-genomics era will further expand our knowledge of metabolic pathways, while advances in systems biology will help us to model the impact of different modifications more accurately. But we should bear in mind that, despite the encouraging results obtained so far, important questions remain largely unanswered to date. For instance, it remains to be shown whether the novel transgenic varieties which showed considerable improvement in certain traits under controlled conditions also outperform conventional varieties under high crop density in the field, something which has rarely been investigated so far.

References

Agius F, Gonzalez-Lamothe R, Caballero JL, Munoz-Blanco J, Botella MA, Valpuesta V (2003) Engineering increased vitamin C levels in plants by overexpression of a D-galacturonic acid reductase. Nat Biotechnol 21:177–181

Al-Babili S, Beyer P (2005) Golden Rice – five years on the road – five years to go? Trends Plant Sci 10:565–573

Altmann T, Felix G, Jessop A, Kauschmann A, Uwer U, Penacortes H, Willmitzer L (1995) Ac/Ds transposon mutagenesis in *Arabidopsis thaliana* – mutant spectrum and frequency of Ds insertion mutants. Mol Gen Genet 247:646–652

An S, Park S, Jeong D-H, Lee D-Y, Kang H-G, Yu J-H, Hur J, Kim S-R, Kim Y-H, Lee M, Han S, Kim S-J, Yang J, Kim E, Wi SJ, Chung HS, Hong J-P, Choe V, Lee H-K, Choi J-H, Nam J, Kim S-R, Park P-B, Park KY, Kim WT, Choe S, Lee C-B, An G (2003) Generation and analysis of end sequence database for T-DNA tagging lines in rice. Plant Physiol 133:2040–2047

ap Rees T, Hill SA (1994) Metabolic control analysis of plant metabolism. Plant Cell Environ 17:587–599

Bailey JE (1991) Toward a science of metabolic engineering. Science 252:1668–1675

Bates PD, Ohlrogge JB, Pollard M (2007) Incorporation of newly synthesized fatty acids into cytosolic glycerolipids in pea leaves occurs via acyl editing. J Biol Chem 282:31206–31216

Beyer P, Al-Babili S, Ye XD, Lucca P, Schaub P, Welsch R, Potrykus I (2002) Golden rice: Introducing the beta-carotene biosynthesis pathway into rice endosperm by genetic engineering to defeat vitamin A deficiency. J Nutr 132:506S–510S

Börnke F, Hajirezaei M, Sonnewald U (2001) Cloning and characterization of the gene cluster for palatinose metabolism from the phytopathogenic bacterium *Erwinia rhapontici*. J Bacteriol 183:2425–2430

Börnke F, Hajirezaei M, Heineke D, Melzer M, Herbers K, Sonnewald U (2002) High-level production of the non-cariogenic sucrose isomer palatinose in transgenic tobacco plants strongly impairs development. Planta 214:356–364

Broun P (2004) Transcription factors as tools for metabolic engineering in plants. Curr Opin Plant Biol 7:202–209

Broun P, Somerville C (1997) Accumulation of ricinoleic, lesquerolic, and densipolic acids in seeds of transgenic Arabidopsis plants that express a fatty acyl hydroxylase cDNA from castor bean. Plant Physiol 113:933–942

Butelli E, Titta L, Giorgio M, Mock HP, Matros A, Peterek S, Schijlen EG, Hall RD, Bovy AG, Luo J, Martin C (2008) Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors. Nat Biotechnol 26:1301–1308

Cahoon EB, Ohlrogge JB (1994) Apparent role of phosphatidylcholine in the metabolism of petroselinic acid in developing Umbelliferae endosperm. Plant Physiol 104:845–855

Cahoon EB, Carlson TJ, Ripp KG, Schweiger BJ, Cook GA, Hall SE, Kinney AJ (1999) Biosynthetic origin of conjugated double bonds: Production of fatty acid components of high-value drying oils in transgenic soybean embryos. Proc Natl Acad Sci USA 96: 12935–12940

Cahoon EB, Hall SE, Ripp KG, Ganzke TS, Hitz WD, Coughlan SJ (2003) Metabolic redesign of vitamin E biosynthesis in plants for tocotrienol production and increased antioxidant content. Nat Biotechnol 21:1082–1087

Cahoon EB, Dietrich CR, Meyer K, Damude HG, Dyer JM, Kinney AJ (2006) Conjugated fatty acids accumulate to high levels in phospholipids of metabolically engineered soybean and *Arabidopsis* seeds. Phytochem 67:1166–1176

Cahoon EB, Shockey JM, Dietrich CR, Gidda SK, Mullen RT, Dyer JM (2007) Engineering oilseeds for sustainable production of industrial and nutritional feedstocks: solving bottlenecks in fatty acid flux. Curr Opin Plant Biol 10:236–244

Dahlqvist A, Stahl U, Lenman M, Banas A, Lee M, Sandager L, Ronne H, Stymne S (2000) Phospholipid:diacylglycerol acyltransferase: an enzyme that catalyzes the acyl-CoA-independent formation of triacylglycerol in yeast and plants. Proc Natl Acad Sci USA 97:6487–6492

Davuluri GR, van Tuinen A, Fraser PD, Manfredonia A, Newman R, Burgess D, Brummell DA, King SR, Palys J, Uhlig J, Bramley PM, Pennings HMJ, Bowler C (2005) Fruit-specific RNAi-mediated suppression of DET1 enhances carotenoid and flavonoid content in tomatoes. Nat Biotechnol 23:890–895

Dehesh K (2001) How can we genetically engineer oilseed crops to produce high levels of medium-chain fatty acids? Eur J Lipid Sci Technol103:688–697
DellaPenna D, Last RL (2006) Progress in the dissection and manipulation of plant vitamin E biosynthesis. Physiol Plant 126:356–368
DellaPenna D, Pogson BJ (2006) Vitamin synthesis in plants: tocopherols and carotenoids. Annu Rev Plant Biol 57:711–738
Durrett TP, Benning C, Ohlrogge J (2008) Plant triacylglycerols as feedstocks for the production of biofuels. Plant J 54:593–607
Dyer JM, Chapital DC, Kuan JW, Mullen RT, Pepperman AB (2002) Metabolic engineering of Saccharomyces cerevisiae for production of novel lipid compounds. Appl Microbiol Biotechnol 59:224–230
Dyer JM, Stymne S, Green AG, Carlsson AS (2008) High-value oils from plants. Plant J 54: 640–655
Fernie AR, Willmitzer L, Trethewey RN (2002) Sucrose to starch: a transition in molecular plant physiology. Trends Plant Sci 7:35–41
Geigenberger P (2003) Regulation of sucrose to starch conversion in growing potato tubers. J Exp Bot 54:457–465
Geigenberger P, Stamme C, Tjaden J, Schulz A, Quick PW, Betsche T, Kersting HJ, Neuhaus HE (2001) Tuber physiology and properties of starch from tubers of transgenic potato plants with altered plastidic adenylate transporter activity. Plant Physiol 125:1667–1678
Geigenberger P, Stitt M, Fernie AR (2004) Metabolic control analysis and regulation of the conversion of sucrose to starch in growing potato tubers. Plant Cell Environ 27:655–673
Grotewold E (2008) Transcription factors for predictive plant metabolic engineering: are we there yet? Curr Opin Biotechnol 19:138–144
Hellwege EM, Czapla S, Jahnke A, Willmitzer L, Heyer AG (2000) Transgenic potato (Solanum tuberosum) tubers synthesize the full spectrum of inulin molecules naturally occurring in globe artichoke (*Cynara scolymus*) roots. Proc Natl Acad Sci USA 97:8699–8704
Henikoff S, Till BJ, Comai L (2004) TILLING. Traditional mutagenesis meets functional genomics. Plant Physiol 135:630–636
Herbers K (2003) Vitamin production in transgenic plants. J Plant Physiol 160:821–829
Hoffmann M, Wagner M, Abbadi A, Fulda M, Feussner I (2008) Metabolic engineering of omega3-very long chain polyunsaturated fatty acid production by an exclusively acyl-CoA-dependent pathway. J Biol Chem 283:22352–22362
Jacobsen JR, Khosla C (1998) New directions in metabolic engineering. Curr Opin Chem Biol 2:133–137
Jako C, Kumar A, Wei Y, Zou J, Barton DL, Giblin EM, Covello PS, Taylor DC (2001) Seed-specific over-expression of an *Arabidopsis* cDNA encoding a diacylglycerol acyltransferase enhances seed oil content and seed weight. Plant Physiol 126:861–874
Jeon J-S, Lee S, Jung K-H, Jun S-H, Jeong D-H, Lee J, Kim C, Jang S, Lee S, Yang K, Nam J, An K, Han M-J, Sung R-J, Choi H-S, Yu J-H, Choi J-H, Cho S-Y, Cha S-S, Kim S-I, An G (2000) T-DNA insertional mutagenesis for functional genomics in rice. Plant J 22:561–570
Jobling S (2004) Improving starch for food and industrial applications. Curr Opin Plant Biol 7:210–218
Jobling SA, Jarman C, Teh MM, Holmberg N, Blake C, Verhoeyen ME (2003) Immunomodulation of enzyme function in plants by single-domain antibody fragments. Nat Biotechnol 21:77–80
Kammerer B, Fischer K, Hilpert B, Schubert S, Gutensohn M, Weber A, Flügge UI (1998) Molecular characterization of a carbon transporter in plastids from heterotrophic tissues: The glucose 6-phosphate phosphate antiporter. Plant Cell 10:105–117
Kim CM, Piao HL, Park SJ, Chon NS, Je BI, Sun B, Park SH, Park JY, Lee EJ, Kim MJ, Chung WS, Lee KH, Lee YS, Lee JJ, Won YJ, Yi G, Nam MH, Cha YS, Yun DW, Eun MY, Han C-d (2004) Rapid, large-scale generation of Ds transposant lines and analysis of the Ds insertion sites in rice. Plant J 39:252–263

Kinney AJ (2006) Metabolic engineering in plants for human health and nutrition. Curr Opin Biotechnol 17:130–138

Knutzon DS, Hayes TR, Wyrick A, Xiong H, Maelor Davies H, Voelker TA (1999) Lysophosphatidic acid acyltransferase from coconut endosperm mediates the insertion of laurate at the sn-2 position of triacylglycerols in lauric rapeseed oil and can increase total laurate levels. Plant Physiol 120:739–746

Kroon JTM, Wei WX, Simon WJ, Slabas AR (2006) Identification and functional expression of a type 2 acyl-CoA : diacylglycerol acyltransferase (DGAT2) in developing castor bean seeds which has high homology to the major triglyceride biosynthetic enzyme of fungi and animals. Phytochemistry 67:2541–2549

Larson TR, Edgell T, Byrne J, Dehesh K, Graham IA (2002) Acyl CoA profiles of transgenic plants that accumulate medium-chain fatty acids indicate inefficient storage lipid synthesis in developing oilseeds. Plant J 32:519–527

Lee M, Lenman M, Banas A, Bafor M, Singh S, Schweizer M, Nilsson R, Liljenberg C, Dahlqvist A, Gummeson PO, Sjodahl S, Green A, Stymne S (1998) Identification of non-heme diiron proteins that catalyze triple bond and epoxy group formation. Science 280:915–918

Mansoor S, Amin I, Hussain M, Zafar Y, Briddon RW (2006) Engineering novel traits in plants through RNA interference. Trends Plant Sci 11:559–565

Metzger JO, Bornscheuer U (2006) Lipids as renewable resources: current state of chemical and biotechnological conversion and diversification. Appl Microbiol Biotechnol 71:13–22

Meyer FD, Talbert LE, Martin JM, Lanning SP, Greene TW, Giroux MJ (2007) Field evaluation of transgenic wheat expressing a modified ADP-glucose pyrophosphorylase large subunit. Crop Sci 47:336–342

Muir SR, Collins GJ, Robinson S, Hughes S, Bovy A, Ric De Vos CH, van Tunen AJ, Verhoeyen ME (2001) Overexpression of petunia chalcone isomerase in tomato results in fruit containing increased levels of flavonols. Nat Biotechnol 19:470–474

Nath U, Becker HC, Möllers C (2006) Increasing erucic acid content in rapeseed. In: OIRA (ed) Oils, fats and lipids for a healthier future, Madrid. Proc Eur Fed Lipid Congr 4:OIRA-003

Ohlrogge J, Browse J (1995) Lipid biosynthesis. Plant Cell 7:957–970

Paine JA, Shipton CA, Chaggar S, Howells RM, Kennedy MJ, Vernon G, Wright SY, Hinchliffe E, Adams JL, Silverstone AL, Drake R (2005) Improving the nutritional value of Golden Rice through increased pro-vitamin A content. Nat Biotechnol 23:482–487

Qi B, Fraser T, Mugford S, Dobson G, Sayanova O, Butler J, Napier JA, Stobart AK, Lazarus CM (2004) Production of very long chain polyunsaturated omega-3 and omega-6 fatty acids in plants. Nat Biotechnol 22:739–745

Regierer B, Fernie AR, Springer F, Perez-Melis A, Leisse A, Koehl K, Willmitzer L, Geigenberger P, Kossmann J (2002) Starch content and yield increase as a result of altering adenylate pools in transgenic plants. Nat Biotechnol 20:1256–1260

Ritsema T, Smeekens SCM (2003) Engineering fructan metabolism in plants. J Plant Physiol 160:811–820

Sayanova O, Haslam R, Venegas Caleron M, Napier JA (2007) Cloning and characterization of unusual fatty acid desaturases from Anemone leveillei: identification of an acyl-coenzyme A C20 Delta5-desaturase responsible for the synthesis of sciadonic acid. Plant Physiol 144:455–467

Schijlen EGWM, Ric de Vos CH, van Tunen AJ, Bovy AG (2004) Modification of flavonoid biosynthesis in crop plants. Phytochemistry 65:2631–2648

Schultz DJ, Suh MC, Ohlrogge JB (2000) Stearoyl-acyl carrier protein and unusual acyl-acyl carrier protein desaturase activities are differentially influenced by ferredoxin. Plant Physiol 124:681–692

Sevenier R, Hall RD, van der Meer IM, Hakkert HJ, van Tunen AJ, Koops AJ (1998) High level fructan accumulation in a transgenic sugar beet. Nat Biotechnol 16:843–846

Shintani D, DellaPenna D (1998) Elevating the vitamin E content of plants through metabolic engineering. Science 282:2098–2100

Shockey JM, Gidda SK, Chapital DC, Kuan JC, Dhanoa PK, Bland JM, Rothstein SJ, Mullen RT, Dyer JM (2006) Tung tree DGAT1 and DGAT2 have nonredundant functions in triacylglycerol biosynthesis and are localized to different subdomains of the endoplasmic reticulum. Plant Cell 18:2294–2313

Smidansky ED, Clancy M, Meyer FD, Lanning SP, Blake NK, Talbert LE, Giroux MJ (2002) Enhanced ADP-glucose pyrophosphorylase activity in wheat endosperm increases seed yield. Proc Natl Acad Sci USA 99:1724–1729

Smidansky ED, Martin JM, Hannah LC, Fischer AM, Giroux MJ (2003) Seed yield and plant biomass increases in rice are conferred by deregulation of endosperm ADP-glucose pyrophosphorylase. Planta 216:656–664

Smidansky ED, Meyer FD, Blakeslee B, Weglarz TE, Greene TW, Giroux MJ (2007) Expression of a modified ADP-glucose pyrophosphorylase large subunit in wheat seeds stimulates photosynthesis and carbon metabolism. Planta 225:965–976

Stark DM, Timmerman KP, Barry GF, Preiss J, Kishore GM (1992) Regulation of the amount of starch in plant tissues by ADP glucose pyrophosphorylase. Science 258:287–292

Storozhenko S, De Brouwer V, Volckaert M, Navarrete O, Blancquaert D, Zhang GF, Lambert W, Van der Straeten D (2007) Folate fortification of rice by metabolic engineering. Nat Biotechnol 25:1277–1279

Suh MC, Schultz DJ, Ohlrogge JB (2002) What limits production of unusual monoenoic fatty acids in transgenic plants? Planta 215:584–595

Sweetlove LJ, Burrell MM, ap Rees T (1996) Characterization of transgenic potato (*Solanum tuberosum*) tubers with increased ADP glucose pyrophosphorylase. Biochem J 320:487–492

Tang GL, Galili G, Zhuang X (2007) RNAi and microRNA: breakthrough technologies for the improvement of plant nutritional value and metabolic engineering. Metabolomics 3: 357–369

Thomaeus S, Carlsson AS, Stymne S (2001) Distribution of fatty acids in polar and neutral lipids during seed development in Arabidopsis thaliana genetically engineered to produce acetylenic, epoxy and hydroxy fatty acids. Plant Sci 161:997–1003

Tjaden J, Möhlmann T, Kampfenkel K, Henrichs G, Neuhaus HE (1998) Altered plastidic ATP/ADP-transporter activity influences potato (*Solanum tuberosum* L.) tuber morphology, yield and composition of tuber starch. Plant J 16:531–540

Tomlinson K, Denyer K (2003) Starch synthesis in cereal grains. Adv Bot Res 40:1–61

van de Loo FJ, Broun P, Turner S, Somerville C (1995) An oleate 12-hydroxylase from *Ricinus communis* l is a fatty acyl desaturase homolog. Proc Natl Acad Sci USA 92:6743–6747

Van Eenennaam AL, Lincoln K, Durrett TP, Valentin HE, Shewmaker CK, Thorne GM, Jiang J, Baszis SR, Levering CK, Aasen ED, Hao M, Stein JC, Norris SR, Last RL (2003) Engineering vitamin E content: from *Arabidopsis* mutant to soy oil. Plant Cell 15:3007–3019

Visser RGF, Somhorst I, Kuipers GJ, Ruys NJ, Feenstra WJ, Jacobsen E (1991) Inhibition of the expression of the gene for granule-bound starch synthase in potato by antisense constructs. Mol Gen Genet 225:289–296

Voelker TA, Worrell AC, Anderson L, Bleibaum J, Fan C, Hawkins DJ, Radke SE, Davies HM (1992) Fatty acid biosynthesis redirected to medium chains in transgenic oilseed plants. Science 257:72–74

Weyens G, Ritsema T, Van Dun K, Meyer D, Lommel M, Lathouwers J, Rosquin I, Denys P, Tossens A, Nijs M, Turk S, Gerrits N, Bink S, Walraven B, Lefebvre M, Smeekens S (2004) Production of tailor-made fructans in sugar beet by expression of onion fructosyltransferase genes. Plant Biotechnol J 2:321–327

Wiberg E, Edwards P, Byrne J, Stymne S, Dehesh K (2000) The distribution of caprylate, caprate and laurate in lipids from developing and mature seeds of transgenic *Brassica napus* L. Planta 212:33–40

Wu GH, Truksa M, Datla N, Vrinten P, Bauer J, Zank T, Cirpus P, Heinz E, Qiu X (2005) Stepwise engineering to produce high yields of very long-chain polyunsaturated fatty acids in plants. Nat Biotechnol 23:1013–1017

Wu L, Birch RG (2007) Doubled sugar content in sugarcane plants modified to produce a sucrose isomer. Plant Biotechnol J 5:109–117

Ye X, Al-Babili S, Ouml KL, Ti A, Zhang J, Lucca P, Beyer P, Potrykus I (2000) Engineering the provitamin A (carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Science 287:303–305

Yu K, McCracken CT, Jr, Li R, Hildebrand DF (2006) Diacylglycerol acyltransferases from *Vernonia* and *Stokesia* prefer substrates with vernolic acid. Lipids 41:557–566

Zhang L, Häusler RE, Greiten C, Hajirezaei MR, Haferkamp I, Neuhaus HE, Flügge UI, Ludewig F (2008) Overriding the co-limiting import of carbon and energy into tuber amyloplasts increases the starch content and yield of transgenic potato plants. Plant Biotechnol J 6:453–464

Zhu C, Naqvi S, Gomez-Galera S, Pelacho AM, Capell T, Christou P (2007) Transgenic strategies for the nutritional enhancement of plants. Trends Plant Sci 12:548–555