3 Current Approaches and Key Applications of Plant Metabolic Engineering

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Abstract

The diversion of carbon flux toward biosynthesis of targeted products could be achieved by manipulation of targeted biosynthesis pathway in plants. This whole process consists of many steps in stepwise manners starting with the identification and isolation of targeted metabolites, elucidation of complete biosynthetic pathway for identification of point of intervention, discovery of corresponding potential metabolic genes, and overexpression of the selected genes in heterologous system and collectively production of the metabolites. The various biochemical processes including transcriptome, translatome, proteome, and reactome are being used to assist metabolic engineering by providing new insights into novel pathways or bottlenecks of existing pathways. Apart from all these, in-depth understanding of metabolic fluxes and feedback regulations is also mandatory for plant metabolic engineering. All these different current approaches are collectively considered for investigating the plant metabolic engineering to understand, reconstruct, analyze, and annotate the targeted pathways. The key applications of plant metabolic engineering have been compiled with a few important applications including improvement of nitrogen utilization in plant, development of highly nutritive food, and generation of biofuel production. In conclusion, the plant metabolic engineering could provide comprehensive evaluation of manipulation of biosynthetic pathways for numerous applications. This compiled information could act as a resource for crop breeding and biotechnology purposes.

Keywords

Plant · Metabolic engineering · Proteins · Metabolites

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Abbreviations

RBS	Ribosomal binding site
NNAAs	Nonnatural amino acids
ORF	Open reading frame
TAG	Triacylglycerol

3.1 Introduction

Metabolic engineering is a process for channeling the carbon flux toward desirable final products through manipulations of selected metabolic pathway to make it more fruitful. Plants synthesize wide range of primary and secondary metabolites for diverse functions in plants. In addition, these diverse wide arrays of compounds are involved in plant growth, development, and adaption during adverse conditions. In continuation, humans utilize major classes of secondary metabolites including isoprenoids, alkaloids and flavonoids for flavors, fragrances, and coloring agents. A range of secondary metabolites has also been utilized for their significant usage in pharmaceutical, nutraceutical, and industrial levels.

After identification of any potential metabolite, the capacity to produce material for industrial and clinical applications is the major limiting factor. The final extraction and purification of a selected metabolite requires extensive analysis from very structurally similar compounds. The final yield of selected metabolites is also defined by particular geographical conditions. In addition, the chemical synthesis of desirable compounds at higher production rate is still challenging for production of selected metabolite with multiple chiral center and labile connectives due to diverse complexity of metabolites. In continuation, optimized synthetic routes become impractical with increasing number of separate steps and subsequently yield decreases (Newhouse et al. 2009).

In addition, to meet the growing demand of value-added metabolites, researchers prefer to develop microbes with varying degrees of success for the efficient and cost-effective products from renewable plant biomass as promising alternative with several advantages. The microbes have also been traditionally exploited for fermentations of foods and feeds including production of organic acids, alcohols, amino acids, and vitamins. The process of microbial production of specific chemicals is also known as more environmentally friendly as compared to chemical synthesis of the same compound. This process also has shorter production times with inexpensive renewable feedstocks. In addition, in contrast to synthetic chemical-based routes, microbial culture can be easily scaled up using fermentation process. A number of easily available relevant organisms including *Escherichia coli*, *Corynebacterium glutamicum*, *Bacillus subtilis*, *Pseudomonas putida*, and *Saccharomyces cerevisiae* can be easily used for tailor-made recombinant strains by recombinant technology for production of a specific value-added metabolite (Du and Shao 2011). The selection of suitable strain development is a major critical step in the optimization of the product processing. The problems associated with

microbial metabolic engineering have been addressed with many strategies including single enzyme change reaction, addition or deletion of existing pathways, transformation of microorganisms into host, or synthetic metabolic pathways (Erb et al. 2017). So, this whole process is suited as a common interdisciplinary framework for the analysis of differential gene expression data with precise information of protein content and in vivo metabolic fluxes. Thus, microbial metabolic engineering utilized principles from chemical engineering, computational science, biochemistry, and molecular biology for production of specific chemicals/metabolites.

Apart from microbial metabolic engineering, plant metabolic engineering has been explored for manipulation of endogenous metabolic pathways in plants/introduction of novel pathway for the production of desirable compound or reduces the level of the undesirable compounds. However potential challenges associated with plant metabolic engineering need to be addressed including elucidation of endogenous pathways for identification of the point of intervention, discovery of most promising metabolic genes, overexpression of gene(s) into heterologous system, and subsequently production of metabolites without harming the targeted plants. The various biochemical processes including transcriptome, translatome, proteome, and reactome are being used to assist metabolic engineering by providing new insights into novel pathways or bottlenecks of existing pathways (Lechner et al. 2017). Investigating biochemical processes also offers the identification of many new plant metabolites (Tobias et al. 2017). In addition, potential bioinformatics tools have also been added to discover the genes for manipulations of selected biochemical pathway (Tatsis and Connor 2016). Apart from all these, in-depth understanding of metabolic fluxes and feedback regulations is also mandatory for plant metabolic engineering. All these different current approaches are collectively considered for investigating the plant metabolic engineering to understand, reconstruct, analyze, and annotate the targeted pathways. In conclusion, in-depth analysis of gene expression pattern, metabolites profiling, and genomic data and their overexpression/silencing provides an effective approach for discovery of gene function for plant metabolic engineering (Prosser et al. 2014; Kagale et al. 2016). The plant metabolic engineering could also be used for comprehensive evaluation of effect of environmental constraints on plant metabolism, and curated information could be used in crop breeding and biotechnology purposes. In addition, plant metabolic engineering has also added new nodes to boost the productivity rate with increased resistance to pathogens (Kumar and Yadav 2017).

In this chapter, we complied the updated knowledge on current approaches and key application of plant metabolic engineering for harnessing the metabolic power of plants for production of metabolites of interest.

3.2 Descriptive Approaches for Plant Metabolic Engineering

A number of diverse approaches can be utilized to quantify the metabolic and regulatory network status including gene expression, protein expression, flux analysis, metabolic concentration, and enzyme activity and collectively considered under

plant metabolic engineering. A number of computational tools can also be useful for integrating and analyzing these datasets for in-depth understanding of plant metabolism (Maudsley et al. 2011; Booth et al. 2013; Jing et al. 2014).

There are three major goals of plant metabolic engineering. The first goal is the accumulation of specific desired compound(s). In contrast, second goal is the least accumulation of targeted undesirable compound(s) and third goal is the biosynthesis of novel compounds. The promising strategies for achieving these three goals reflect the engineering of identified regulatory steps in a selected biosynthetic pathway resulted into diversion of metabolic flux for targeting specific metabolite(s) (Pickens et al. 2011; Moses et al. 2013). Sometimes, it is also necessary to completely block the parallel competitive pathways or introduce shortcuts to divert metabolic pathway in a particular direction as given in Fig. 3.1. However, the strategies, which only include the manipulation of single rate-limiting step, are often nullify by the system itself to maintain the homeostasis (Curien et al. 2009), so that targeting multiple steps in same or competitive pathways could divert the metabolic pathway in a more predictable and productive manner. A brief representation for targeting multiple pathways is given in Fig. 3.1. Moreover, "omic" era has covered cellular and subcellular pathways and provided the evidence that metabolic pathways can be controlled at multiple levels rather than step-by-step manner. In addition, recombinant DNA technology has also offered opportunities for improving or increasing the metabolic flux by means of genetic manipulation in biosynthetic pathways (Adrio and Demain 2010). In addition, some strategies might call for the transient expression of any foreign construct for investigating the interaction between selected gene(s) and gene product(s), while other strategies are based upon the generation of transgenic plants expressing a new gene product for many years.

With these collectively evidences, the metabolic engineering has shifted away from targeting individual gene/pathway to manipulate entire cell itself. Synthetic biology has the potential to fabricate regulatory networks throughout the cell by using heterologous genes, gene deletions, and overexpression-related strategies (He

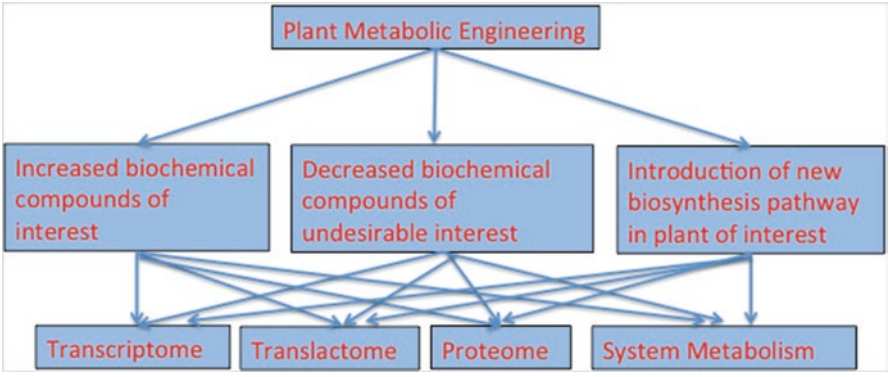

Fig. 3.1 Basic strategy for manipulation pf metabolite of interest in the plants

et al. 2017). High-throughput DNA sequencing techniques have also facilitated the discovery of unknown gene clusters and cryptic pathways that are responsible for the production of engineered products in microorganisms (Luo et al. 2016). Now next-generation sequencing-generated datasets have also been used for construction of novel pathways for manipulation purposes (Luo et al. 2016).

3.2.1 Engineering at Transcriptome and Translatome Level

The extensive progress has been carried out in analyzing cellular functions with measurement of the cellular components including genes, mRNA, proteins, and metabolites. These collectively studies have given key tools for investigation and deciphering mechanism of the cellular activities. The cell-wide measurements involving quantifying genome-wide total mRNA levels show a significant promise for designing of predictive models. Thus, among numerous high-throughput tools, genome-wide transcriptional profiling is extensively used. Transcriptome analysis helps to calculate the total cellular mRNAs in terms of their level and identification of up- and downregulated genes. In continuation, the most promising strategy to get the desirable metabolites profile can be carried out by manipulating the regulation of targeting RNA transcripts. The mRNA(s) level of specific target(s) can play a regulatory role by altering mRNA structure for synthesis and folding of protein (Faure et al. 2016). A number of factors including promoter strength, mRNA stability, and gene copy number have been documented affecting mRNA stability. To counter this constraint, synthetic promoters and optimized transcription factors have been utilized to control the mRNA expression profile. The inability to predict precisely gene expression levels interrupts the metabolic engineering of biological systems. Kosuri et al. (2013) documented more than 12,000 combinations of common promoters and ribosomal binding sites and measured DNA, RNA, and protein levels. The same study also allowed the quantification of global effects including influence of translation rate on mRNA stability and effect of mRNA secondary structure on translation rate and emphasized the importance of screening of synthetic libraries for desired behavior. Gonzalez-Ramos et al. (2008) compared a specific strain to be resistant to ethanol with another strain with ethanol overproduction. After a careful investigation, a number of sets of differentially expressed genes have been identified from multiple branches of metabolism. Thus the most powerful strategy to identify a target for metabolic engineering through gene expression analysis is to analyze a collection of strains. On the basis of these studies, the transcriptional information could be harness for design of cell factories. The genome-wide transcriptome analysis could be exploited for generation of desired strain using metabolic engineering. In addition, transcriptome analysis also offers the possibility of elucidating global regulatory processes. The whole genome mRNA profiles can also help to identify key genetic trends that may be important consideration for understanding of cellular flux and regulations. In conclusion, transcriptional analysis also provides one more additional level of control to manipulate the flux network.

Thus, it has been proved that variations in mRNA and proteins levels are most prevalent in prokaryotic expression (Payne 2015). To understand the relationship between mRNA and protein abundance, synthetic biology has been implemented with combination of 12,500 promoters and ribosomal binding site in *E.coli* (Kosuri et al. 2013). The sevenfold change in protein abundance has been observed by manipulating the small nucleotide space around ribosomal binding site in *Saccharomyces cerevisiae*. Translation efficiency has been also documented which depends upon mRNA secondary structure (Sugimoto et al. 2015). Additional studies are also required to understand expression of different types of protein classes through mRNA secondary structure formation.

The codon usage bias is believed to be the most influential factor affecting translation efficiency. However, these codon biases disrupt the mRNA stability and translational rates but act as essential motivation for gene manipulation. Transgenic expressions have become burden for eukaryotes by either upregulating or downregulating the normal routine cellular activity. On the other hand, there is a great need for identification and development of bacterial host for synthetic codon adaption strategies that design multiprevailing parameters. Recently, a condition-specific codon optimization approach has been created to improve heterologous protein levels of bacterial genes in yeast (Lanza et al. 2014). Other combinatorial libraries have been developed for translational efficiency and protein integrity. This approach provided synonymous codon for a representative protein in native and non-native hosts, and these are translated that are subsequently responsible for co-translational folding (Makino et al. 2011).

In addition, to find the detailed description of cellular response to specific manipulation, a number of studies reflected integration of two or more omic responses simultaneously. So, proteomics, protein-protein interactions, and protein-DNA interactions have also been integrated to elucidate regulatory phenomena. The high-throughput analytical approaches have been utilized for metabolic engineering. An integration of data obtained from genome-wide transcript level with in vivo fluxes provided reconstruction of genome-scale model to characterize growth and regulation through major carbon metabolism in *S. cerevisiae* (Karhumaa et al. 2005).

3.2.2 Engineering at Proteome Level

It has also been established that the manipulation at proteome level also acts as a regulatory level for metabolic engineering. Both protein and metabolic engineering are synergistic and work together to build organisms for efficient production of compounds of interest. To make an efficient strategy, there is a mandatory requirement to divert the resources from growth and unwanted metabolic pathways toward desired pathway with maximum carbon flux. Thus, a regulatory component in this scheme is the engineering of proteins via facilitating carbon flux. The protein engineering involves many strategies including altering protein structure, targeted or

random mutagenesis, to obtain functional perturbations such as decreased product inhibition, with improved substrate sensitivity, higher catalytic rates, selection of specific cofactor, and reduced substrate competition. Collectively, all these modifications boost the titers and yield of metabolically produced compounds. However, a single protein or pathways is totally unable to manipulate their behavior significantly due to complexity of regulation processes even in simplest bacterial expression system.

The protein manipulations for metabolic engineering depend upon the cell growth selection and assay-based screens for identification of protein using direct evolution strategy. In addition, several methods have been adopted for random and targeted genetic diversity for generation of protein libraries. Some of them are error-prone PCR (polymerase chain reaction), target and random mutagenesis, chemical mutagens, and DNA shuffling. In addition, engineering of transcription factor has also been used for more effective manipulations in selected pathways. Apart from chemical mutagenesis, another promising method for multigenic modifications for complex change of phenotype is the mutation of selected transcription factor. The major advantage of this method is that expression of all coordinated genes is altered simultaneously by mutagenesis of only one single protein. However, the engineering of transcription factor is much easier in eukaryotic cells than prokaryotic cells because of involvement of zinc finger protein for assisting the binding of transcription factor with regulating site of DNA (Hudson and Ortlund 2014). Zhang et al. (2012) proposed a similar strategy in which global transcription factor cyclic AMP receptor of *E.coli* has been used for improvement of 1-butanol tolerance. The incorporation of nonnatural amino acids into proteins has also been explored as a powerful tool for protein engineering. However, there are no direct examples for use of NNAAs (nonnatural amino acids) for metabolic engineering, but they offer potential advantages for metabolic engineers. Leonard et al. (2010) adopted the combined strategy of metabolic and protein engineering of a terpenoid biosynthetic pathway for overproduction of levopimaradiene. This study highlights the importance of protein engineering with metabolic pathway as a key strategy in achieving microbial biosynthesis and production of key metabolites.

Enzyme engineering also plays a key role by creation of new functions of enzyme, shaping enzyme specificity, deletion of competitive reactions, and adding new node of novel enzyme (Broadwater et al. 2002). It provides not only the starting point to the reactions for novel enzyme functionalities but also provides native activities for increased and higher productivity (Yoshikuni et al. 2008). To identify promiscuous enzyme activity is a major challenge in enzyme engineering. To counter this challenge, the Biochemical Network Integrated Computational Explorer (BNICE) has sorted out many existing enzyme chemistries for novel enzyme mechanism (Hatzimanikatis et al. 2005). It also predicts novel enzyme/function based on physicochemical substrate/product, enzyme similarity based on enzyme classification, and novel enzyme within biosynthetic pathway (Jacobson et al. 2014). A total of 20 heterologous pathways have also been predicted in *E. coli* (Campodonico et al. 2014).

3.2.3 System Metabolic Engineering

The construction and probing of large-scale datasets are basic requirement for improving or synthesizing a cellular function in the form of metabolic models. To access cellular and metabolic function, large-scale global measurements are the basic requirement. In continuation, wealth of data generated through genomic (from genetic materials), transcriptomic (by mRNA profiling), and proteomic (protein profile) and fluxomic data (flux analysis) has been required for development of cell model that is an invaluable tool for metabolic engineering. A rational prediction of different phenotypically changes has been observed with manipulation of media composition, gene knockouts, and incorporation of heterologous/novel pathways. The cell-wide flux maps have been used as first attempt of system biology toward metabolic engineering. However, now measurement of transcript levels, protein levels, interactions between different proteins/metabolites, concentrations, and even localizations of proteins has also been considered for building of comprehensive cell models. However, till today most comprehensive and predicted functional models of system biology are global metabolic network reconstructions, which serve as interpretation of material balance and stoichiometric reactions occurring within the cell and predicted many metabolic perturbations correctly. In conclusion, in silico genome-scale metabolic reconstructions behave as baseline for applications of system metabolic engineering.

There are many reports proving that system metabolic engineering had significant successes. The production of L-valine and L-threonine has been improved in *E. coli* using similar approaches (Park et al. 2007; Lee et al. 2013). Both reports used advantages of transcriptome analysis and in silico model-based metabolic reconstruction for the identification of particular gene knockouts. In continuation, the single-, double-, and triple-gene knockouts have been identified for improved lycopene using a genome-scale metabolic model of *E.coli*. Previously, Lee et al. (2013) reported 85% of the maximum theoretical yield of succinic acid using metabolic reconstruction in *E. coli*. Beyond *E. coli*, *S. cerevisiae* has also been exploited for improvement of succinic acid. An in silico genome-scale metabolic reconstruction of *S. cerevisiae* has been reported for strain improvement (Chung et al. 2010). All these reports provided the supportive evidences for the power of in silico modeling for manipulation of certain biosynthetic pathway and metabolites. However, the selection of a platform organism acts as a critical decision for any metabolic engineering. The most common model organisms are *S. cerevisiae* and *E. coli*, and many steps are required for any non-model organisms for in silico genome-scale metabolic reconstructions. The major requirement for system biology is to access the whole genome information of selected organism(s) to determine innate cellular capacities for in silico genome-scale metabolic reconstruction(s). The genomic exploration allows the comparative genomics in between genomes across different strains/species and can be exploited for rational manipulations of metabolic pathways by identification of rate-limiting steps/enzymes to improve the yield of metabolite of interest. After gaining genomic information, the next step is the discovery of all unique ORF coding sequences for enzymes of selected metabolic network, and

all identified ORFs are assigned based upon their respective enzyme functionality. In continuation, coupling can also facilitate the process of genome annotation and metabolic reconstructions with metabolic databases including KEGG, LIGAND, BioCYC, etc. All these databases have repository of bioinformatics and systems biology knowledge sets for all elucidated pathways in a system. All these complied information can be helped to create the metabolic network and component interactions. After construction of metabolic network, flux analysis can be performed using in silico metabolic reconstruction to assess its accuracy. The most effective approach has been the use of flux analysis to model gene knockouts in metabolic reconstructions in order to improve the yield by either deletion or overexpression approach. However, construction of cell-wide flux analysis using carbon-labeled substrates is the first attempt in implication of system biology toward metabolic engineering. However, global metabolic network reconstruction is the most comprehensive and predictive model of system biology that can be utilized for metabolic engineering. For optimization of the production of the chemical of interest, the detailed understanding of the network and distribution of flux are also necessarily required. These tools have also been utilized extensively for metabolic engineering because these allow the detailed exploration of the structure and design of metabolic network that represents the biochemistry of organisms. The stoichiometric methods, which are based on the collected biochemical knowledge surrounding a specific metabolic network of an organism of interest, could help construct metabolic models based on annotated genome sequence. Such model helps to perform simulation based upon on all available information about all reactions of a metabolic network using information about the stoichiometry of the network as inputs and predict metabolic states of organism under particular conditions.

In conclusion, the system metabolic engineering mainly depends upon the accuracy of high-throughput data for construction of in silico models. However, the system metabolic engineering for model organisms with already established metabolic reconstructions helped to discover gene knockout for improvement of product yield.

3.3 Key Applications of Plant Metabolic Engineering:

Over the last few decades, great success has been achieved by plant metabolic engineering to improve productivity of crops (Lau et al. 2014; Long et al. 2015). Synthetic biology and golden rice are two major notable success stories in the field of metabolic engineering that not only increase the productivity but also improve the nutritional value (Lau et al. 2014). In this chapter, a few long standing challenges with their applications have been discussed. A series of discussions for improvement of nitrogen utilization in plants, generation of crops with high nutritional value, alternative source of fuel production, and improving carbon fixation by enhancing photosynthetic efficiency have also been presented. These are examples of future emerging areas in the field of metabolic engineering.

The first example is the improvement of nitrogen utilization in plants by utilizing metabolic engineering. Nitrogen is a fascinating micronutrient element in biological systems with many unique properties. In agriculture field, nitrogen is used as a fertilizer to get a better yield. It has been estimated that a total of 180 million tons of synthetic nitrogen are utilized as fertilizer worldwide. However, a huge consumption of nitrogen becomes a disaster agent for soil, aquatic system, and environment. There are two possible ways by which plants can be engineered to fulfill their own demand of nitrogen consumption. These two possible approaches are biological nitrogen fixation and symbiotic nitrogen fixation. The biological nitrogen fixation is carried out by nitrogenase with conversion of nitrogen into ammonia. Interestingly, this enzyme is not present in plant system; thus plants cannot use nitrogen instead of ammonia. The biological fixation phenomenon is reported in bacteria named as diazotrops, which occurred into the soil and catalyze the conversion of nitrogen into a more bioavailable form, ammonia. Root nodules of plants further take the reduced form of ammonia. However, disruption of the NifA/NifL (nitrogen fixation activator/nitrogen regulatory gene that inhibits the activation of other genes by NifA protein)-mediated ammonia regulation of *nif* (nif regulon) gene expression and inhibition of GS (glutamine synthase) or GOGAT (glutamine oxoglutarate aminotransferase) for deficient ammonium assimilation are associated with improvement of ammonium excretion in diazotrops. The first approach was carried out by either overexpression of *NifA* or deletion of *NifL* in *Azotobacter vinelandii* (Ortiz-Marquez et al. 2012). By using metabolic engineering, the improvement of nitrogen utilization in plants can also be achieved. The nitrogen assimilation is carried out by enzyme glutamine synthetase (GS) via the GS/GOGAT cycle. This GS/GOGAT pathway is a metabolic node with a regulatory position in plant amino acid metabolism. The nitrogen assimilation starts with nitrogen compounds in soil and 2-oxoglutarate carbon skeletons. The transgenic plant lines with improved nitrogen assimilation and improved growth have been achieved by overexpressing of a number of transgenes and transcription factors. Similarly studies have also been reported with the overexpression of gene-encoded GS enzyme which indicated a number of changes in plant metabolism (CÁNovas et al. 2006). The re-assimilation of ammonium has been achieved in lotus deficient in plastidic isoform of glutamine synthase (Pérez-Delgado et al. 2016). The overexpression/knockout mutation of GOGAT has also reported with better grain filling and improved biomass (CÁNovas et al. 2006).

Plants are the major source for human nutrition and have also been targeted for improvement via metabolic engineering approach. The energy supply as well as sugars/starch determines the nutritional quality of crops. The potato is the most important food (non-cereal) crop, which is deficient in the sulfur-containing amino acids (methionine and cysteine). The metabolic engineering could offer to manipulate the targeted amino acid biosynthesis for improvement of nutritive value of potato (Stiller and Dancs 2007). Similarly, Giuliano et al. (2008) reviewed all potential attempts toward the manipulation of the carotenoid biosynthesis in plants. Carotenoids including B-carotene, zeaxanthin, and astaxanthin have been documented for beneficial effects for human health. In plants, carotenoids are derived by isoprenoid precursors from MEP pathway (2-C-methyl-D-erythritol 4-phosphate). The MEP pathway is the

main pathway for the biosynthesis of carotenoids, tocopherols, certain sesquiterpenes, monoterpenes, and others. The phytoene synthase is a promising key step for increasing carotenoid biosynthesis in plants. Overexpression of the phytoene synthase encoded gene in plants has been exploited to improve various carotenoid contents. A β-carotene enriched crop products offers an alternative to fight with vitamin A deficiency in infants and adult. The highest β-carotene levels have been reported for different "golden" staple crops including maize, rice, wheat, and potato (Ye and Bhatia 2012). Even the engineering of carotenoids content in leaf tissue has been reported for improved stress resistance. Metabolic engineering also leads the way to manipulating plant lipid composition (Napier et al. 2014). The manipulation of plant seed oil by improving fatty acid composition has long been an objective of metabolic engineering. The metabolic engineering is targeting oil-related traits in oilseed crops that are a promising source of food as well as fuel. However, the development of seed transcriptome is necessary to elucidate the functional seed-specific metabolic pathway. The seed-specific RNAi suppression of genes encoded fatty acid desaturase 2 (FAD2, control desaturation of oleic acid) and fatty acid elongase 1 (FAE1, elongation to C20 and C22 chain lengths) in *Camelina sativa* which has been achieved with altered composition of seed lipid (Nguyen et al. 2013). Phenylpropanoids are also key metabolites of nutritional diet due to their nature of antioxidants. The alteration of composition of novel wax esters in the seeds, part of transgenic *C. sativa* using metabolic engineering, has also been documented (Ruiz-López et al. 2012, 2017). The most explored pathway for secondary metabolic pathway is phenylpropanoid pathway, which is a main target for plant metabolic engineers to improve the content of secondary metabolites. An example of this attempt is to be presented in which the manipulation of phenylpropanoid pathway has been carried out in tomato by introducing transcription factor encoded by *AtMYB12* (Pandey et al. 2015). However, *AtMYB12* transcription factor has also been exploited for diversion of the route of carbon flux toward aromatic amino acid biosynthesis for improving phenylpropanoids level (flavonoids and hydroxycinnamates) (Pascual et al. 2016). In conclusion, all these selected examples provided evidence that metabolic engineering is an effective approach that not only improves nutritional value but also increases the commercial value of crops. However, elevated level of a specific nutrient compound could alter the taste and flavor of commercial part(s) of plants. For example, steviol glycosides and related esters impart distinctive to strawberry plants (Brandle and Telmer 2007).

Another promising application is the transition toward a biofuel-based economy using metabolic engineering. Presently, bioethanol is a major biofuel in huge demand that is produced by sugars of sugarcane and corn. The another source is the production of bioethanol from lignocellulose biomass which originated from residual biomass of crops (wheat, corn, and sugarcane) or biomass (poplar and switch grass) from crops. However, biomass form poplar and switchgrass can also be a good source for fuel production. In contrast, the direct accessibility of the polysaccharides of biomass for enzyme degradation is directly influenced by the cellulose content and requires one more preliminary additional step of hydrolysis either under acidic or alkaline conditions. Thus, metabolic engineering offered to limit the lignin content in plants because it is a limiting factor for production of fermentable sugars.

Van Acker et al. (2014) documented the downregulation of *cinnamoyl-CoA reductase* which is linked with improvement of ethanol production. In another attempt, overexpression of *monolignol ferulate transferase* (*MFT*) in transgenic poplar plants has been documented with increase of monolignol ferulate conjugates that helps to make cell wall more susceptible to chemical polymerization (Wilkerson et al. 2014). Yang et al. (2013) proposed a systems-wide approach for manipulation of cell wall biosynthesis using metabolic engineering in plants. Authors documented enhanced lignin biosynthesis in the vessels with improved saccharification yields while maintaining biomass. In continuation, an attempt to increase the secondary wall thickening by using an artificial positive feedback back loop has also been documented. This was carried out using a combinatorial approach in which a transcription factor encoded by *NST1* was also overexpressed with higher content of released sugar. This approach could be an effective approach for enhanced bioethanol production using crop plants. Another approach is the utilization of TAG (triacylglycerols), which acts as energy-rich form of biofuels. Thus, the strategy to increase the content of TAGs in vegetative tissues also offers the improvement of accessibility to biofuels. In continuation, all genes involved into elevated TAG levels have been identified (Vanhercke et al. 2014). The silencing of a gene encoded enzyme ADP-glucose pyrophosphorylase that is involved in starch biosynthesis was documented with diversion of carbon away from starch and toward TAG biosynthesis (Rismani-Yazdi et al. 2011). Silencing of gene-encoded enzyme, peroxisomal ABC transporter1 (PXA1), is documented with lesser oxidation of fatty acid in the mitochondria (Boisnard et al. 2009). Zale et al. (2016) documented the metabolic engineering of sugarcane plants with overexpression of *WRINKLED1*, *DGAT1-2*, and *OLE1* genes as well as simultaneously silencing of *AGPase* and *PXA1* that accumulated higher amount of triacylglycerols (TAGs) as compared to control plants.

In conclusion, metabolic engineering offers opportunity for the accumulation of novel fatty acids for improving agronomical traits. However most of metabolic engineering of oil-related traits used *Arabidopsis* as a testing plant for identification of genes for improving the oil production. Documented the accumulation of omega-3 LC-PUFA (long-chain polyunsaturated fatty acids) and DHA (docosahexaenoic acid) in *Arabidopsis*. In addition, *Camellia sativa* offers a model plant for metabolic engineering because the transformation has been easily achieved using *Agrobacterium*-mediated floral infiltration method. The accumulation of EPA (eicosapentaenoic acid and DHA) was documented in *Camelina sativa* with equivalent levels as found in fish oils (Ruiz-Lopez et al. 2017). Another plant, crambe (*Crambe abyssinica*), is also known as a dedicated industrial oil crop and could be proved as the best model crop for metabolic engineering of oil traits in seeds.

Thus, the metabolic engineering of LC-PUFAs in transgenic *Camelina* seeds as well erucic acid in transgenic crambe seeds provided the evidences of successful manipulations of fatty acid traits in oilseed crops. The effective strategy for generating appropriate level of oil in vegetative tissues has also been proposed by using integrated approach of coordinated overexpression of engineered transcription factor (linked with upregulation of fatty acid) encoded by genes, and genes encoded TAG biosynthetic enzymes simultaneously with downregulation of TAG catabolic enzymes.

References

Adrio J-L, Demain AL (2010) Recombinant organisms for production of industrial products. Bioeng Bugs 1:116–131

Boisnard S, Espagne E, Zickler D, Bourdais A, Riquet AL, Berteaux-Lecellier V (2009) Peroxisomal ABC transporters and β-oxidation during the life cycle of the filamentous fungus *Podospora anserine*. Fungal Genet Biol 46:55–66

Booth SC, Weljie AM, Turner RJ (2013) Computational tools for the secondary analysis of metabolomics experiments. Comput Struct Biotechnol J 4:e201301003

Brandle JE, Telmer PG (2007) Steviol glycoside biosynthesis. Phytochemistry 68:1855–1863

Broadwater JA, Whittle E, Shanklin J (2002) Desaturation and hydroxylation. Residues 148 and 324 of Arabidopsis FAD2, in addition to substrate chain length, exert a major influence in partitioning of catalytic specificity. J Biol Chem 277:15613–15620

Campodonico MA, Andrews BA, Asenjo JA, Palsson BO, Feist AM (2014) Generation of an atlas for commodity chemical production in Escherichia coli and a novel pathway prediction algorithm, GEM-path. Metab Eng 25:140–158

Cánovas FM, Gallardo F, Jing ZP, Pascual MB (2006) Transgenic approaches to engineer nitrogen metabolism. In: Fladung M, Ewald D (eds) Tree transgenesis. Springer, Berlin/Heidelberg

Chung BKS, Selvarasu S, Camattari A, Ryu J, Lee H, Ahn J, Lee H, Lee D-Y (2010) Genome-scale metabolic reconstruction and *in-silico* analysis of methylotropic yeast *Pichia pastoris* for strain improvement. Microb Cell Factories 9:50

Curien G, Bastien O, Robert-Genthon M, Cornish-Bowden A, Cárdenas ML, Dumas R (2009) Understanding the regulation of aspartate metabolism using a model based on measured kinetic parameters. Mol Syst Biol 5:271

Du J, Shao Z (2011) Engineering microbial factories for synthesis of value-added products. J Ind Microbiol Biotechnol 38:873–890

Erb TJ, Jones PR, Bar-Even A (2017) A. Synthetic metabolism: metabolic engineering meets enzyme design. Curr Opin Chem Biol 37:56–62

Faure G, Ogurtsov AY, Shabalinam SA, Koonin EV (2016) Role of mRNA structure in the control of protein folding. Nucleic Acids Res 44:10898–10911

Giuliano G, Tavazza R, Diretto G, Beyer P, Taylor MA (2008) Metabolic engineering of carotenoid biosynthesis in plants. Trends Biotechnol 26:139–145

Gonzalez-Ramos D, Cebollero E, Gonzalez R (2008) A recombinant *Saccharomyces cerevisiae* strain overproducing mannoproteins stabilize wine against protein haze. Appl Environ Microbiol 74:5533–5540

Hatzimanikatis V, Li C, Ionita JA, Henry CS, Jankowski MD, Broadbelt LJ (2005) Exploring the diversity of complex metabolic networks. Bioinformatics 21:1603–1609

He F, Murabito E, Westerhoff H (2017) Synthetic biology and regulatory: where metabolic systems biology meets control engineering. J R Soc Interface 13:20151046

Hudson WH, Ortlund EA (2014) The structure, function and evolution of proteins that bind DNA and RNA. Nat Rev Mol Cell Biol 15:749–760

Jacobson MP, Kalyanaraman C, Zhao S, Tian B (2014) Leveraging structure for enzyme function prediction: methods, opportunities and challenges. Trends Biochem Sci 39:363–371

Jing LS, Shah FFM, Mohamad MS, Hamran NL, Mohamed S, Debris S, Alashwal H (2014) Database and tools for metabolic network analysis. Biotechnol Bioprocess Eng 19:568–585

Kagale S, Nixon J, Khedikar Y, Pasha A, Provart NJ (2016) The developmental transcriptome atlas of the biofuel crop *Camelina Sativa*. Plant J 88:879–894

Karhumaa K, Hahn-Hagerdahl B, Gorwa-Grauslund MF (2005) Investigation of the limiting metabolic steps in the utilization of xylose by recombinant *Saccharomyces cerevisiae* using metabolic engineering. Yeast 22:359–368

Kosuri S, Goodman DB, Cambray G, Mutalik VK, Gao Y, Arkin AP, Endy D, Church GM (2013) Composability of regulatory sequences controlling transcription and translation in *Escherichia coli*. Proc Natl Acad Sci U S A 110:14024–14029

Kumar V, Yadav SK (2017) Transgenic tobacco overexpressing dihydroflavonol reductase and anthocyanidin reductase showed improved flavan-3-ols contents and tolerance against biotic and abiotic stress conditions. 3Biotech 7:177

Lanza AM, Curran KA, Rey LG, Alper HS (2014) A condition-specific codon optimization approach for improved heterologous gene expression in *Saccharomyces cerevisiae*. BMC Sys Biol 8:33

Lau W, Fischbach MA, Osboun A, Sattely ES (2014) Key applicatons of plant metabolic engineering. PLoS Biol 12:e1001879

Lechner A, Brunk E, Keasling JD (2017) The need for integrated approaches in metabolic engineering. Cold Spring Harb Perspect Biol 8:a023903

Lee JH, Jung S-K, Bui LM, Kang KH, Song J-J, Kim SC (2013) Improved production of L-threonine in *Escherichia* coli by use of a DNA scaffold system. Appl Environ Microbiol 79:774–782

Leonard E, Ajikumar PK, Thayer K, Xiao W-H, Mo JD, Tidor B, Stephanopoulos G, Prather KLJ (2010) Combining metabolic and protein engineering of a terpenoid biosynthetic pathway for overproduction and selectivity control. Proc Natl Acad Sci U S A 107:13654–13659

Long SP, Marshall-Colon A, Zhu X-G (2015) Meeting the global food demand of the future by engineering crop photosynthesis and yield potential. Cell 161:56–66

Luo Y, Enghiad B, Zhao H (2016) New tools for reconstruction and heterologous expression of natural product biosynthetic gene clusters. Nat Prod Rep 33:174–182

Makino T, Skretas G, Georgiou G (2011) Staining engineering for improved expression of recombinant proteins in bacteria. Microb Cell Fac 10:32

Maudsley S, Chadwick W, Wang L, Zhou Y, Martin B, Park S-S (2011) Bioinformatic approaches to metabolic pathways analysis. Methods Mol Biol 756:99–130

Moses T, Pollier J, Thevelein JM, Goossens A (2013) Bioengineering of plant (tri)terpenoids: from metabolic engineering of plants to synthetic biology *in vivo* and *in vitro*. New Phytol 1:27–43

Napier JA, Haslam RP, Beaudoin F, Cahoon EB (2014) Understanding and manipulating plant lipid composition metabolic engineering leads the way. Curr Opin Plant Biol 19:68–75

Newhouse T, Baran PS, Hoffmann RW (2009) The economics of synthesis. Chem Soc Rev 38:3010–3021

Nguyen HT, Silva JE, Podicheti R, Macrander J, Yang W, Nazarenus TJ, Nam JW, Jaworski JG, Lu C, Scheffler BE (2013) Camelina seed transcriptome: a tool for meal and oil improvement and translational research. Plant Biotechnol J 11:759–769

Ortiz-Marquez JCF, Nascimento MD, de los Dublan M, Curati A (2012) Association with an ammonium-excreting bacterium allows diazotrops culture of oil-rich eukaryotic microalgae. Appl Environ Microbiol 78:2345–2352

Pandey A, Misra P, Choddhary D, Yadav R, Goel R, Bhanbhani S, Sanyal I, Trivedi R, Trivedi PK (2015) AtMYB12 expression in tomato leads to large scale differential modulation in transcriptome and flavonoid content in leaf and fruit tissues. Sci Rep 5:12412

Park JH, Lee KH, Kim TY, Lee SY (2007) Metabolic engineering of Escherichia coli for the production of L-valine based on transcriptome analysis and *in-silico* gene knockout simulation. Proc Natl Acad Sci U S A 104:7797–7802

Pascual MB, El-Azaz J, de la Torre FN, Cañas RA, Avilla C, Cánovas (2016) Biosynthesis and metabolic fate of phenylalanine in conifers. Front Plant Sci 7:1030

Payne SH (2015) The utility of protein and mRNA correlation. Trends Biochem Sci 40:1–3

Pérez-Delgado CM, García-Calderón M, Márquez AJ, Betti M (2016) Reassimilation of photorespiratory ammonium in *Lotus japonicus* plants deficient in plastidic glutamine synthetase. PLoS One 11:e0156568

Pickens LB, Tang Y, Chooi Y-H (2011) Metabolic engineering for the production of natural products. Annu Rev Chem Biomol Eng 2:211–236

Prosser GA, Larrouy-Maumus G, de Carvalho LPS (2014) Metabolic strategies for the identification of new enzyme functions and metabolic pathways. EMBO Rep 15:657–669

Rismani-Yazdi H, Haznedaroglu BZ, Bibby K, Peccia J (2011) Transcriptome sequencing and annotation of the microalgae *Dunaliella tertiolecta*: pathway description and gene discovery for production of next-generation biofuels. BMC Genomics 12:148

Ruiz-López N, Haslam RP, Venegas-Calerón M, Li T, Bauer J, Napier JA, Sayanova O (2012) Enhancing the accumulation of omega-3 long chain polyunsaturated fatty acids in transgenic *Arabidopsis thaliana* via iterative metabolic engineering and genetic crossing. Trans Res 21:1233–1243

Ruiz-Lopez N, Broughton R, Usher S, Salas JJ, Haslam RP, Napier JA, Beaudoin F (2017) Tailoring the composition of novel wax esters in the seeds of transgenic *Camelina sativa* through systematic metabolic engineering. Plant Biotechnol 15:837–849

Stiller I, Dancs G (2007) Increasing the nutritive value of potato by metabolic engineering of cysteine content. Acta Aliment 37:103. https://doi.org/10.1556/AAlim.2007.0021

Sugimoto Y, Vigilante A, Darbo E, Zirra A, Militti C, D'Ambrogio A, Luscombe NM, Ule J (2015) hiCLIP reveals the *in-vivo* atlas of mRNA secondary strcutures recognized by Staufen 1. Nature 519:419–494

Tatsis EC, Connor SEO (2016) New developments in engineering plant metabolic pathways. Curr Opin Biotechnol 42:126–132

Tobias JE, Jones PR, Bar-Even A (2017) Synthetic metabolism: metabolic engineering meets enzyme design. Curr Opin Chem Biol 37:56–62

Van Acker R, Leple J-C, Aerts D, Storme V, Goeminne G, Ivens B, Legee F et al (2014) Improved saccharification ans ethanol yield form field-grown transgenic poplar deficient in cinnamoyl-CoA redcutase. Proc Natl Acad Sci U S A 111:845–850

Vanhercke T, Tahchy AE, Liu Q, Zhou X-R, Shrestha P, Divi UK, Ral J-P, Mansour MP et al (2014) Metabolic engineering of biomass for high energy density: oilseed-like triacylglycerol yields from plant leaves. Plant Biotechnol J 12:231–239

Wilkerson CG, Mansfield SD, Lu F, Withers S, Park JY, Karlen SD, Gonzales-Vigil E, Padmakshan D, Unda F, Rencoret J et al (2014) Monolignol ferulate transferase introduces chemically labile linkages into the lignin backbone. Science 344:90–99

Yang F, Mitra P, Zhang L, Prak L, Verhertbruggen Y, Kim JS, Sun L, Zheng K, Tang K, Auer M, Scheller HV, Loque D (2013) Engineering secondary cell wall deposition in plants. Plant Biotechnol J 11:325–335

Ye VM, Bhatia SK (2012) Metabolic engineering strategies for the production of beneficial carotenoids in plants. Food Sci Biotechnol 21:1511–1517

Yoshikuni Y, Dietrich JA, Nowroozi FF, Babbitt PC, Keasling JD (2008) Redesigning enzymes based on adaptive evolution for optimal function in synthetic metabolic pathways. Chem Biol 15:607–618

Zale J, Jung JH, Kim JY, Pathak B, Karan R, Liu H, Chen X, Wu H, Candreva J, Zhai Z, Shanklin J, Altpeter F (2016) Metabolic engineering of sugarcane to accumulate energy-dense triacylglycerols in vegetative biomass. Plant Biotechnol J 14:661–669

Zhang H, Chong H, Ching CB, Song H, Jiang R (2012) Engineering global transcription factor cyclic AMP receptor protein of *Escherichia coli* for improved 1-butanol tolerance. Appl Microbiol Biotechnol 94:1107–1117