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SHORT COMMUNICATION



An outlook on metabolic pathway engineering in crop plants

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ABSTRACT

To produce the essential secondary metabolites, plants are the major and important target source materials for conducting the high-profile metabolic engineering studies. Metabolic pathway engineering of both microorganism targets and plants target contribute towards important drug discovery. In order to efficiently work out in advanced plant metabolic pathway engineering techniques, a detailed knowledge and expertise is essentially needed regarding the plant cell physiology and the mechanics of plant metabolism. Mathematical and statistical models to scale and map the genome for integrative metabolic pathway activity, signal transduction mechanism in the genome, gene regulation and the networks of protein-protein interaction can provide the in-depth knowledge to work efficiently on plant metabolic pathway engineering studies. Incorporation of omics data into these statistical and mathematical models is crucial in the case of drug discovery using the plant system. Recently, artificial intelligence concept and approaches are experimentally applied for efficient and accurate metabolic engineering in plants.

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INTRODUCTION

The science of metabolic engineering is defined as the redirection of one or more enzymatic reaction processes to produce new compounds in an organism, improve the rate of production of existing chemical compounds or mediate the compound degradation. The plant metabolic engineering is quite a young science to investigate the metabolic pathways in plants. Attempts to use this knowledge to engineer metabolic processes in plants, it requires the basic molecular biological and biotechnological tools and expertise techniques such as gene cloning, promoter analysis in the gene construct, protein targeting protocols, plant transformation principles and other areas of plant biochemical genetics. To procure the desired cell phenotypes, metabolic engineering has improved a lot by redirecting appropriate metabolic signalling fluxes with the help of metabolic flux analysis protocols (Stephanopoulos *et al.*, 1993; Bailey *et al.*, 2002).

In the era of post-genomics, a huge volume of metabolites data, protein interactions data and gene analysis data, introduces more accurate analytical techniques to explore and understand cellular metabolism which reduces the costs of oligonucleotide synthesis exponentially. In recent years, the plant metabolic engineering has emerged with various novel analytical approaches, such as, gene deletion, heterologous genes expression in plant system and identification of tools to control gene expression in plants or plant cell regulation (Blazeck *et al.*, 2010; Tyo *et al.*, 2007). Application of metabolic engineering systems to microbial strain such as *Escherichia coli* and *Saccharomyces cerevisiae*, enables metabolic engineers to overproduce the essential secondary metabolites for both industrial and pharmaceutical use (Kim *et al.*, 2016). Although different kinetic models of the parts of such pathways have been developed, the study on signalling, gene regulation and protein-protein interaction networks are still in nascent stage. Such genome-scale models will enable metabolic engineers to analyse the complex

pathways which are integrated with each other, generally leading to rational design of new vision to the traditional metabolic pathway analysis (Abernathy *et al.*, 2017). However, lack of detailed knowledge to demonstrate pathway models is a major limitation factor in plant metabolic pathway engineering (Krambeck *et al.*, 2005; Umaña *et al.*, 1997). Thus, metabolic engineers need both in silico models for prediction, screening directed evaluation and experimental analysis for suggesting novel hypotheses and confirming in silico predictions in plant system (Boyle *et al.*, 2012) using the recent metabolic engineering tools.

Requirements for metabolic engineering strategies development

Metabolic engineering is an emerging biological science tool which is having immense contributions towards agriculture, medical science and basic science. Plant metabolic engineering has been emerged as an advanced technology for future biological archives, which provides strategies to utilize renewable natural resources to generate alternative biofuels (Schuhmann *et al.*, 2012). Genome-scale models of integrated metabolic techniques, signal transduction mechanisms, protein-protein interaction, regulatory genetic networks and advanced tools for gene editing have pushed plant metabolic engineering a few steps ahead. However, such tools essentially need detailed investigation about cellular properties at molecular level, cellular pathway regulations dynamics, enzyme kinetics, gene-protein interaction and relationships among each other's. Computational analysis based on multiple omics data with the use of recent advances in artificial intelligence science in plants, can also facilitate such efficient and optimal metabolic engineering strategies in plant system. In case of crop plants, enhancement of nitrogen supply, nutrient uptake, biofuel production, photosynthesis proficiency and disease prevention can be achieved through advanced metabolic engineering strategies. Such strategies and advanced methods of metabolic engineering on microorganism as well as plants lead to successful drug design for various complex diseases frequently affecting the plant system. In this context of experiment, the NET analysis (Network-embedded thermodynamic analysis) and TMFA (Thermodynamics-based metabolic flux analysis) have been widely used (Kümmel *et al.*, 2006; Zamboni *et al.*, 2008; Olson *et al.*, 2015). Finally, the consistent integrated pathway model qualifies for computational and statistical analysis which leads to metabolic control analysis (MCA) in plants with the support of the science of artificial intelligence.

Metabolic pathway engineering in plant

Plant metabolic engineering advances in various aspects rapidly over last few decades. Golden rice (Ye *et al.*, 2000; Tang *et al.*, 2009) is a significant example of metabolic engineering in plants. Here, metabolic engineers face great challenges including self sufficient plant creation in case of nitrogen requirement, enhancement of the nutrient in crop plants, biofuel production from plants, photosynthetic efficiency improvement and plant

disease control. Nitrogen fertilizers damage the soil, environment and nearby water resources during the application to crop plants for the yield advancements, when the crop is on field. Thus, it will be a great advantage, if a plant can manage its own nitrogen requirement by itself without depending on chemical fertilizers. Nitrogenase, an enzyme, generally found in some bacteria, converts atmospheric nitrogen into biologically available form ammonia. Plants are able to produce and manage the required nitrogen, if the genes of nitrogenase enzymes are expressed in plant host system. However, metabolic engineers are able to express only eight genes for nitrogenase in plant host till date (Kebeish *et al.*, 2007), which is a big future challenge for the researchers for further investigation.

It is also essential to determine the tissue location and cell type, where the gene constructs for nitrogenase needs to be expressed (Dasgupta *et al.*, 2020). Metabolic engineering strategies contribute an exponential role in enhancing the nutrient contents in crop plant species. The targets for this context are the metabolic pathways, producing phytoalexins and flavonoids, which are helpful mostly in the chemo-preventive mechanism of horticultural crop plants. An innovative plant breeding technology is recently reported, which fortified the crop broccoli with enhanced content of the glucosinolate glucoraphanin (Armah *et al.*, 2013), consumption of which reduces the risk of cancer in human beings. It was also observed that, the flavonoid anthocyanin has been enhanced three-fold by expressing two transgenes from snapdragon in tomato plant (Butelli *et al.*, 2008), which is a good sign for future research.

Some investigations show that, how iodine content in *Arabidopsis thaliana* can be enhanced by expressing NIS sodium-symporter gene of human thyroid gland and knocking out *HOL-1* gene. Apart from golden rice, the nutrient content in maize and wheat can also be enhanced using the metabolic engineering (Morris *et al.*, 2006). Recent finding of metabolic engineering shows that DHA (Docosapentaenoic acid) can be produced from *Camelina sativa* by introducing a transgenic *D6-desaturase* gene from yeast (Dasgupta *et al.*, 2020). In this scenario, new genome editing tools like ZFNs (zinc finger nucleases), TALENs (transcription activator-like effector nucleases) and CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats-CRISPR-associated gene 9), play predominant role in plant genome metabolic pathway engineering (Shan *et al.*, 2013; Li *et al.*, 2015).

Rapid increase in world population and urbanization gives rise to the importance of improving the photosynthetic efficiency of plant. The C4 and CAM (Crassulacean acid metabolism) photosynthetic system are available to develop more efficient and sophisticated genome editing or transformation methods for improving the photosynthetic efficiency of plant (Dasgupta *et al.*, 2020). Metabolic engineering of biosynthetic pathways contributes to generate plant chemical defence compounds with antimicrobial properties, generally useful in crop protection studies (Collinge *et al.*, 2016). Commercially grown transgenic crops, such as maize, cotton and soybean are more tolerant to herbicides, such as glyphosate, and resistant to insect pests.

They can successfully be produced by transferring a single gene (for example, endotoxin encoding genes from *Bacillus thuringiensis*).

Multiple gene transfer technique is still under microscopic investigation and analysis. The most extensive metabolic engineering strategy for plant disease control is to introduce *resveratrol synthase*, and *stilbene synthase* genes (Delaunois et al., 2009). Here, different kinds of enzymes, involved in the biosynthesis of a specific defence compound can be encoded from the non-homologous genes, contained in the gene cluster. In this context, ten candidate genes are identified by a biosynthetic gene cluster for the anti-tumour alkaloid noscapine found in *Papaver somniferum* (Winzer et al., 2012). Besides all these, gene silencing may arise due to expression of multiple genes of a pathway simultaneously. These studies may help to investigate the complex metabolic pathways in crop plants in future.

Role of artificial intelligence in plant metabolic pathway engineering

In order to predict appropriate and significant target genes for plant metabolic pathway dynamics, the role of machine learning techniques is essentially need. Efficient and optimal system metabolic engineering now a days is driven by high throughput transcriptomics, proteomics and metabolomics data mining and analysis (Kim et al., 2020; Dasgupta et al., 2020). Consequently, modern machine learning algorithms including deep learning approaches can facilitate metabolic engineers to analyse these bio big data, helps in predicting the efficient pathways for metabolic engineering. A deep learning-based technique *DeepRibo*, based on convolutional neural networks and recurrent neural network, is invented for gene annotation in prokaryotes efficiently, without any help of gene homology analysis (Clauwaert et al., 2019). *DeepEc* (Ryu et al., 2019) is another convolutional neural networks-based method with homology analysis for predicting enzyme using protein sequences as inputs.

In the field of metabolic pathway reconstruction, a machine learning based method *3N-MCTS* (Segler et al., 2018) has been developed, which is a retro synthesis method based on integration of three distinct artificial neural networks. The goal of this technique is to explore efficient synthetic routes to produce a target molecule from a host plant species. In addition, convolutional neural networks combined with linear regression models (Groher et al., 2018; Carbonell et al., 2018) can be used to help in optimizing plasmid copy number and selecting promoter region which can be helpful in plant transformation experiments. Recently, an advanced deep learning-based method *DeepCRISPR* (Chuai et al., 2018) is capable of predicting on-target to be knocked out and off-target sites of single-guide RNAs efficiently. Even now a days automated processes using robots (Boles et al., 2017; Chao et al., 2017) in plant systems metabolic engineering are much more needed. However, in order to utilize machine learning techniques and artificial intelligence efficiently, careful planning is required to generate high quality datasets with standard data format, data type and content (Zampieri et al.,

2019). In addition, the predictions from machine learning techniques should be appropriately validated through appropriate experimental mechanistic models to get into the insights of biological metabolic processes deeply, for proper investigation (Dasgupta et al., 2020).

Conclusion

Plant metabolic engineering enables pharmaceutical scientists in developing preventive medicines or strategies to combat chronic human diseases by utilizing higher number of phytonutrients in fruit and vegetables. For these purposes, metabolic engineers have focused on up or down regulation of required genes to redistribute steady state fluxes of specific metabolic pathways. Recently, dynamic regulation strategies can rebalance metabolic fluxes depending on the alteration of cell phenotypes. Moreover, *CRISPR-Cas9*, *ZFNs* and *TALNs* genome editing tools has revolutionized genome editing strategy in crop plants. In addition, exploration of interactions among different metabolic, gene regulatory, signal transduction and protein-protein interaction networks, drive engineers to employ more sophisticated and advanced technology for improved metabolic engineering in near future. However, plant metabolic engineers need more effective computational models, with the support of artificial intelligence, integrating metabolic, signalling, gene regulatory and protein-protein interaction networks, mainly in eukaryotic cells, together detailed system knowledge including proper regulatory guidelines.

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