Vol. 4 Issue- 2, October 2023 (e-ISSN: 2582-8223)

Role of Metabolic Engineering in Plants for Biofuel Production

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ARTICLE ID: 54

Abstract

Increase in fossil fuels for transportation and other industrial use leads to adverse impact on environment, sustainability and rise in price. This leads to find an alternative fuel derived from agricultural residues. Agricultural residues are rich source of cellulose; hemicellulose and lignin. These biomasses are recalcitrant to separate into individual components. The pretreatment of the biomass can be carried out by various physical and chemical methods convert them into simpler sugars. These simple sugars serve as a source for production of ethanol during fermentation process. Various other products derived through fermentation process are n-butanol, hydrocarbons, biochemical and platform chemicals used in pharmaceutical and other industries. Algae and oleaginous organisms are another feedstock explored for fuel production. This popular article reviews the principle, role and applications of synthetic biology in altering the metabolic pathway in plants for the production of biofuel.

Introduction

Due to increase in fossil fuels for transportation and industrial applications leads increase in greenhouse gasses. Utilization of fossil fuel releases 30 billion metric tons of carbon di oxide annually worldwide. This results in the increase in the global temperature and causes adverse effect on the environment. To overcome the extensive usage of fossil fuel, it can be replaced by renewable energy sources because of its sustainability and eco-friendly characteristics. The fuel derived through plant and microorganisms are termed as Biofuel. It represents alternative to fossil fuels. The first-generation biofuels are bioethanol and biodiesel derived from food materials like sugarcane, corn, potatoes, beet and vegetable oil. The usage of these food materials leads to food security issues and disturbs the human food chain.

The alternatives to first generation biofuel are derived from algae, agricultural and forest residues. These residues contain lignocellulosic materials from which fermentable sugars are extracted for the fermentation process to yield ethanol. The recalcitrant nature of the lignocellulosic biomass makes it difficult to extract the sugars. This can be overcome by physical and chemical pretreatment of biomass. Ethanol produced from biomass can be blend with fossil fuels in order to operate normal engines. In addition to ethanol various other products like n-butanol, short chain alcohols, alkanes and alkenes are also produced. The emerging area in biotechnology, synthetic biology plays an important role in production of biofuels from non-food materials through the metabolic alterations in plants and microorganisms. Synthetic biology has ultimate goals of being able to design and build engineered biological systems that can process information, produce biochemicals and energy, provide food and maintain and enhance human health and the environment (2). It employs engineering principles and biological tools to engineer biological system. The engineering of biological systems has enormous potential to reshape the world in a variety of areas, including the sustainability of all systems capable of manufacture at both macro- and micro-levels, as well as addressing various issues in health and general medicine. The important role of synthetic biology in the production of biofuel lies in the improvement of recalcitrant biomass and cellulose content, engineering efficient enzymes to hydrolyze the biomass, altering the microorganisms to accumulate more lipid contents and to alter the metabolic pathways for economically valuable products.

Metabolic Engineering

Metabolic engineering, is an important application of synthetic biology, is mainly used to optimize genetic and regulatory process within the cell to increase the production of desired substances. In metabolic engineering, the current focus is to target the regulatory networks in a cell so as to efficiently engineer the metabolism. Many biorefineries are currently being built across the globe to test and refine technologies for the production of biofuels and chemicals from renewable biomass, which will help in reducing “greenhouse gas” emissions (5). Potential biofuels produced from metabolically engineered microbes includes short-chain alcohols and alkanes to replace gasoline, fatty acid methyl esters (FAMEs), fatty alcohols, fatty acids, and isoprenoid-based biofuels to replace diesel oil. Other products derived through metabolic engineering are artemisinic acid in engineered yeast, *n*-butanol in *S. cerevisiae*, fatty acid-derived biofuels in *E. coli* and the modulation of metabolic flux using synthetic protein frameworks scaffolds.

Role of Synthetic Biology in Feedstock Improvement

Lignocellulosic biomass formed from the waste portion of a plant biomass in the form of agricultural, industrial, domestic and forest residues, energy crops such as cambu napier grass that can be grown on marginal lands, has the potential to become an alternative source for fuels production. The various pretreatment processes are required to achieve the complete hydrolysis of lignocellulosic biomass. The lignocellulosic biomass consists basically of cellulose, hemicelluloses, lignin and a small amount of pectin. Cellulose and hemicelluloses, are hydrolyzed to yield sugars and undergo fermentation to produce biofuels. The efficiency of the hydrolysis, depends largely on the enzymes hydrolyzing the biomass. Among the cell wall components, lignin serves as barrier in accessing the enzymes for hydrolysis. Reducing the proportion of lignin in the cell wall and modifying its structure can results in easy degradation, accessibility to enzymes and reducing the cost of biomass processing.

Engineering of plant that produce less amount of lignin can be done by modulating the key enzymes involved in the lignin biosynthesis. The down regulation of six genes involved in the lignin biosynthetic pathway using antisense approach in transgenic Alfalfa. The suppression of genes involved in the early stages was found to be most effective in reducing the lignin content. The extent of cellulose-mediated digestion of the untreated stems from two classes of transgenic Alfalfa was comparable to that observed for the digestion of lignin-free, microcrystalline cellulose. The down regulation of hydroxycinnamoyl-CoA:NADPH oxidoreductase (CCR) in poplar (*Populus*) was found to result in a more digestible cellulose by *Clostridium cellulolyticum* and produced twice the amount of fermentable sugar.

The down regulation of hydroxycinnamate-CoA/5-hydroxyferuloyl-CoA-ligase in transgenic *Populus tremuloides* resulted in a 45% decrease in lignin content and 15% increase in cellulose. This is mainly due to the quantitative or qualitative changes in one cell-wall component can often result in changes to other cell-wall components. Decreases in lignin content have also been reported by the down regulation of phenyl ammonia lyase (PAL), which is the key enzyme responsible for the downstream regulation of the lignin biosynthesis flux. Down regulation of 4-Hydroxycinnamate 3-hydroxylase led to change in the structure of lignin. This increase in the content of p-hydroxyphenyl units relative to the normally dominant G:S ratio. Another study showed the down regulation of cinnamyl alcohol dehydrogenase in poplar results in increase in less conventional syringyl units and β-O-4-bonds and large number of

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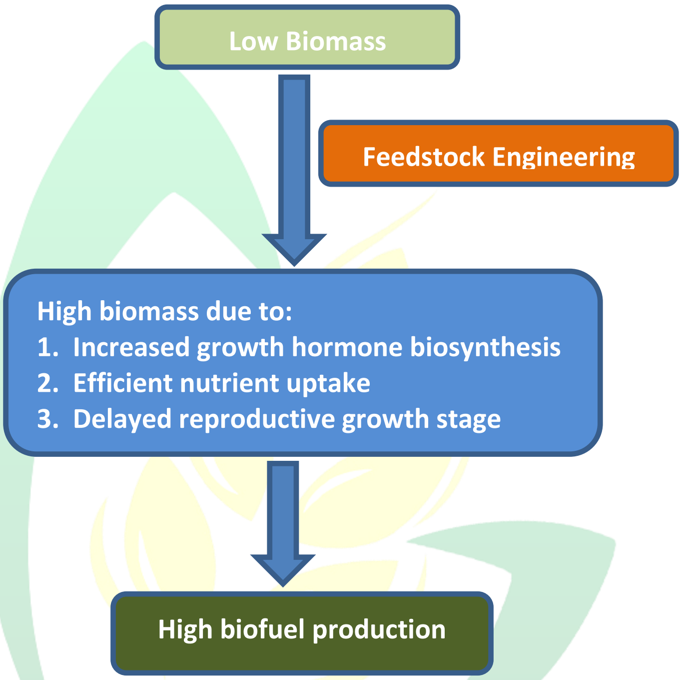
free phenolic groups. Lignin by nature contains ether bonds that are difficult to degrade and with the help of metabolic engineering ester bonds are introduced into lignin backbone which can be easily broken down by chemically.

Feedstock Engineering for Increased Biomass

Another important approach for feedstock engineering is focused on enhancing the polysaccharide production that, eventually, leads to an increase in the overall crop biomass.



An increased biomass will increase the availability of raw materials and, ultimately, decrease the cost of biofuel production. Over expression of floral repressor gene causes an increase in vegetative growth stage in *Arabidopsis thaliana*. Transformation of this gene in tobacco delayed flowering stage by three weeks and significantly increased transgenic plant biomass at lab scale.

The plant biomass growth can be increased by regulating the growth regulators like brassinosteroids and gibberellins. Fructose-1, 6-bisphosphatase plays an important role in CO_2 assimilation and coordinating carbon and nitrogen metabolism to increase sucrose production. This gene is down regulated in Arabidopsis to increase the production of sucrose. The biomass in plants can be increased through the availability of key nutrients. Phosphorous is an important nutrient for photosynthesis, respiration and regulation of many enzymes. The expression of acid phosphatase in Arabidopsis resulted in a twofold increase in biomass production when supplied with phytate as phosphorous source in soil. The expression of biomass hydrolyzing enzymes in plants can improve for the extraction of monomeric sugars for ethanol production. Thermostable enzyme endo glucanase from *Acidothermus cellulyticus* has been expressed in rice and corn stovers to convert into glucose. Enzyme Engineering leads to higher catalytic and specific activity in enhancing the hydrolysis of lignocellulosic biomass. The expression of bifunctional and multifunctional enzymes increases the efficiency in the hydrolysis of biomass.

Summary:

Metabolic engineering in plants through synthetic biology can improve the characteristics of the biomass composition and ease in breakdown of the bonds which interlinks the biomolecules present in the cell wall during pretreatment process. The various biofuels produced through this technology are methanol, ethanol, n-butanol, propanol and biochemicals. The fossil fuel shortage and increase in the price of fuel can results in the alternative route for the fuel production. The biofuel produced through the metabolic engineering in plants will be beneficial for the future needs in transportation and energy production.

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