Plant Science 178 (2010) 61–72

Contents lists available at ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

Review

Plant cell wall reconstruction toward improved lignocellulosic production and processability

Miron Abramson [a], Oded Shoseyov [a,b], Ziv Shani [b,*]

[a] The Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture, and the Otto Warburg Minerva Center for Agricultural Biotechnology, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel
[b] CBD Technologies Ltd. Pekeris 2, P.O. Box 199, Rehovot 76100, Israel

ARTICLE INFO

Article history:
Received 24 June 2009
Received in revised form 11 November 2009
Accepted 13 November 2009
Available online 24 November 2009

Keywords:
Biofuels
Cellulosic ethanol
Lignocellulosics
Saccharification
Cell wall modification

ABSTRACT

Natural resources and environmental quality are in constant decline in parallel with the rapid growth of the world population. Current methods of energy consumption are considered environmentally hazardous and contribute to global warming. To address this rapidly growing concern, development of improved extraction techniques for renewable fuel alternatives is underway. To date, most bioethanol is derived from conventional food and animal feed crops such as corn and sugarcane. A mass shift towards second generation lignocellulosic biomass-derived biofuels will rely on techniques that will enable easy access to plant cell wall components. Lignocellulosic conversion to biofuel requires pretreatment with heat and acid to break the cell wall for effective lignin release. Transgenic plants have been designed for such processes to express reduced or modified lignin for facilitation of saccharification. Plants have been successfully engineered to express enhanced levels of glycoside hydrolases to enable efficient and cost-effective polysaccharide hydrolysis. Furthermore, strategies focusing on increased accessibility of cellulose-degrading enzymes to their substrate have been developed, and rely on cell wall-targeted expression of glycoside hydrolases, cellulose binding modules (CBMs) or other cell wall modifying proteins. A novel approach to cell wall modification comprising the introduction of non-crystalline, soluble polysaccharides to cell walls is discussed. Such approaches will further advance efforts toward establishment of applicable and effective biofuel production methods.

Contents

* Corresponding author. Tel.: +972 8 9319550; fax: +972 8 9319515.
E-mail address: zshani@cbd-tech.com (Z. Shani).

0168-9452/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.plantsci.2009.11.003

1. Introduction: the increasing importance of lignocellulosic biomass

While many strategies and technologies have been proposed to curb the deterioration of environmental quality or to offer alternative energy sources, no ultimate solution has been developed thus far. In an attempt to encourage development of creative solutions to prevent the approaching crisis, the former Saudi Arabian oil minister warned that "The Stone Age didn't end for lack of stone, and the oil age will end long before the world runs out of oil" [1].

Biomass as biofuel, the world's earliest source of energy, has provided a means of heating, illumination and cooking for centuries. The plant can be viewed as a solar energy collector, as it shifts light energy to simple sugars through photosynthesis. Combustible and highly energetic polymers are then formed upon CO_2 fixation, and generate a composite cell wall constituted of cellulose, hemicelluloses and lignin [2].

To date, most ethanol fuel has been generated from corn grain or sugar cane, also referred to as "first generation" feedstocks. Bioconversion of such crops to biofuel competes with food production for land, has a high feedstock cost and replace only a small proportion of fossil fuel production. The main challenges associated with development of "second generation" biomass-derived biofuels include maximization of biomass yield per hectare per year, maintenance of sustainability while minimizing agricultural inputs and prevention of competition with food production. With these considerations in mind, much focus has been placed on conversion of lignocellulosic biomass to fermentable sugars. Ultimately, lignocellulosic-derived ethanol has the potential to meet most of global transportation fuel needs with much less impact on food supply, with lower agricultural inputs and less net carbon dioxide emissions than fossil fuels (Table 1) [3,4].

However, due to the complex structure of plant cell walls, cellulosic biomass is more difficult to break down into sugars than starch. In general, lignocellulosic biomass feedstock is made up of complex structures that are mainly comprised of cellulose, hemicellulose and lignin. The amount of each component, the ratio between them and the type of the hemicellulose is largely dependent on the feedstock type [5]. This composite provides plant cell walls with strength and resistance to degradation and is critical to many physiological functions [6]. However, polymeric lignin prevents access of enzymes and chemicals to hemicellulose and cellulose, reducing the degradability of the carbohydrate material. The degree of lignification as well as cellulose crystallinity and extent of its polymerization [7] are among the factors believed to contribute to the recalcitrance of lignocellulosic feedstock to saccharifying chemicals or enzymes. Thus, the main technological barrier to employment of lignocellulosic biomass as a source of energy is the effective breakdown of the polysaccharide wall into fermentable sugars, to enable a maximal fuel per unit biomass yield [8].

The conversion of lignocellulosic biomass to bioethanol is a three step process that involves a pretreatment stage followed by saccharification of cellulose and hemicellulose to simple sugars via hydrolysis and then fermentation of the free sugars to ethanol. The leading lignocelluloses feedstock candidates today are corn stover and rice straw, as well as perennial grasses, such as switchgrass, *miscanthus* and woody lignocelluloses such as poplar and eucalyptus. To adapt each crop as a feedstock for industrial processing, ideally its cell wall composition needs to be resolved in order to optimize it by breeding and genetic engineering for maximal value. The differences and advantage of each crop have been previously discussed and reviewed [9–11]. The pretreatment phase is characterized by removal of crosslinking bonds within the cell wall through several methods: heat and acid treatment, steam explosion or ammonia-fiber explosion of plant fibers residue. These methods have been previously summarised [12]. The heat/acid-based pretreatment is the most researched, promising and close to commercial realization [13]. The pretreatment is crucial for full access of hydrolytic enzymes to cellulose and hemicellulose. Aside from the costliness of these methods, the pretreatment phase produces toxic byproducts such as acetic acid and furfurals that subsequently inhibit hydrolytic enzymes and fermentation [13]. Enzymatic hydrolysis is the enzymatic conversion of the cell wall polysaccharides to fermentable sugars. In order to make enzymatic hydrolysis process cost effective, further improvement is required for the feedstock accessibility and for the pretreatment. The fermentation process is limited due to two main factors: toxic substances are increased by the pretreatment process and by the limited number of sugar types which can be fermented by microorganisms.

Commercial realization of biofuel production requires an integrated solution combining appropriate biofuel crops, biomass modification techniques and sophisticated process engineering. Application of recent advances in plant genetic engineering, carbohydrate chemistry and increased knowledge of plant cell wall ultrastructure toward reengineering crop cell walls, can assist in reduction of biomass saccharification and conversion costs, while significantly increasing biofuel production yields. This review will discuss the most recent plant cell wall modifications and plant engineering technologies to improve sugar yields upon heat/acid pretreatment and reduce energy demands and cost of the bioethanol production process.

Table 1
Cellulosic ethanol: goals and impacts.[a].

	Past-starch ethanol	Starch and cellulosic ethanol	Future prediction-cellulosic ethanol
Liter (billion)	15	75	100 to 750+
Fossil fuel displacement	2%	10%	15 to 100%
CO_2 reduced	1.8%	9%	14 to 90%

[a] Source: Data reproduced from the U.S. Department of Energy Office of Science report [3].

2. Modification of cell wall polysaccharides

2.1. Cell wall polysaccharide synthesis and disassembly

Understanding the synthesis and breakdown of the cell wall composite frame will help to effectively manipulate its polymers to get a more amenable biofuel crop. The major components of plant biomass or lignocelluloses are cellulose, hemicellulose, lignin and also pectin, which is more abundant in dicots [14]. The (1-4)-β-linked glucose cellulose polymer is the most abundant polysaccharide on earth. Still, the control of its biosynthesis is not completely understood. The cell wall polysaccharides are synthesized along various pathways and within different subcellular subcompartments. Briefly, cellulose is synthesized by plasma membrane-associated, hexagonal shaped complexes that each contain 36 cellulose synthase modules (CesA) [15,16]. Upon its synthesis, cellulose is assembled and integrated within the cell wall via close physical interactions with hemicelluloses, enzymatic modifications, and chemical crosslinking. A highly compact lattice of microfibrils is assembled and linked via hydrogen bonds to form crystalline cellulose. These microfibrils serve as a scaffold for the deposition of other wall components, such as hemicellulose and pectin [17,18].

In contrast to cellulose, hemicellulose and pectin synthesis occurs in the Golgi bodies [19]. Hemicellulosic polysaccharides are complex molecules that crosslink neighboring cellulose micro-