

# Plants to power: bioenergy to fuel the future

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**Bioenergy should play an essential part in reaching targets to replace petroleum-based transportation fuels with a viable alternative, and in reducing long-term carbon dioxide emissions, if environmental and economic sustainability are considered carefully. Here, we review different platforms, crops, and biotechnology-based improvements for sustainable bioenergy. Among the different platforms, there are two obvious advantages to using lignocellulosic biomass for ethanol production: higher net energy gain and lower production costs. However, the use of lignocellulosic ethanol as a viable alternative to petroleum-based transportation fuels largely depends on plant biotechnology breakthroughs. We examine how biotechnology, such as lignin modification, abiotic stress resistance, nutrition usage, *in planta* expression of cell wall digestion enzymes, biomass production, feedstock establishment, biocontainment of transgenes, metabolic engineering, and basic research, can be used to address the challenges faced by bioenergy crop production.**

## Multiple choices for bioenergy

Bioenergy refers to renewable energy from biological sources that can be used for heat, electricity and fuel, and their co-products. There has been a resurgence of interest in bioenergy recently, and several articles have already addressed the potential impact of biotechnology on renewable energy [1–5]. However, in this review we will integrate several of the key components of bioenergy, including feedstock, processing platforms, enabling biotechnologies, ecological effects and economics, to gauge how plant biotechnology might impact bioenergy efficiency and sustainability. We will discuss the crucial choices of feedstock (e.g. starch, sugar, fatty acid or cellulose) and energy product (e.g. ethanol, biodiesel and others), the economic feasibility and the pros and cons of different choices, and the major technical breakthroughs needed to develop a sustainable bioenergy industry.

## Choices of platforms

In terms of modern bioenergy, ethanol, biodiesel and biogas are the three major bioenergy products. Ethanol and biodiesel can be used as transportation fuels, and ethanol

is also an important raw product in the chemical industry. Therefore, ethanol production has a particularly important role in transforming petroleum-based economies to biomass-based sustainable and environment-friendly economies.

## Ethanol processing platforms

Ethanol can be produced using agricultural products such as starch and sugar, or lignocellulosic biomass (see Glossary; Figure 1a and b). Currently >10 billion gallons of ethanol is produced globally per year from starch (maize) and sugar (sugarcane and sugar beet) through mature industrialized procedures, including hydrolysis of starch and fermentation of sugar (Figure 1a) [3,6]. Starch and sugar-based ethanol is often referred to as a first-generation biofuel.

## Glossary

**Agriculture residuals (or residues):** straw or 'stover' that are left in the field after harvest, or forest product 'waste' such as woodchips.

**Bioenergy feedstock:** either the biomass crops themselves or the raw material that is input into the biorefinery.

**Biofuel cells:** various electrochemical systems that can generate a current with the electron or proton donated from microorganisms, often through oxidation reaction.

**Carbon balance:** also known as carbon dioxide balance; calculated as carbon dioxide emitted by biomass production and usage subtracted from the carbon dioxide fixed in the plant material, both above ground and underground. Thus, a negative carbon balance is desirable.

**Fermentation:** the conversion of sugars into ethanol by microorganisms under anaerobic conditions.

**Greenhouse effect (also known as global warming):** worldwide rise in temperature caused by particular gases, such as methane and carbon dioxide, trapping heat from the sun on the Earth's surface. These gases are therefore called 'greenhouse gases'.

**Lignocellulosic biomass:** plants grown for ethanol production using the entire aboveground biomass. 'Lignocellulosic' refers to the plant biomass that is composed of cellulose, hemicellulose and lignin polymers. Biomass can be hydrolyzed and resulting sugars can be used for ethanol production and potentially other biofuels.

**Net energy balance (NEB):** the difference between the energy output and the energy input for biomass production and processing.

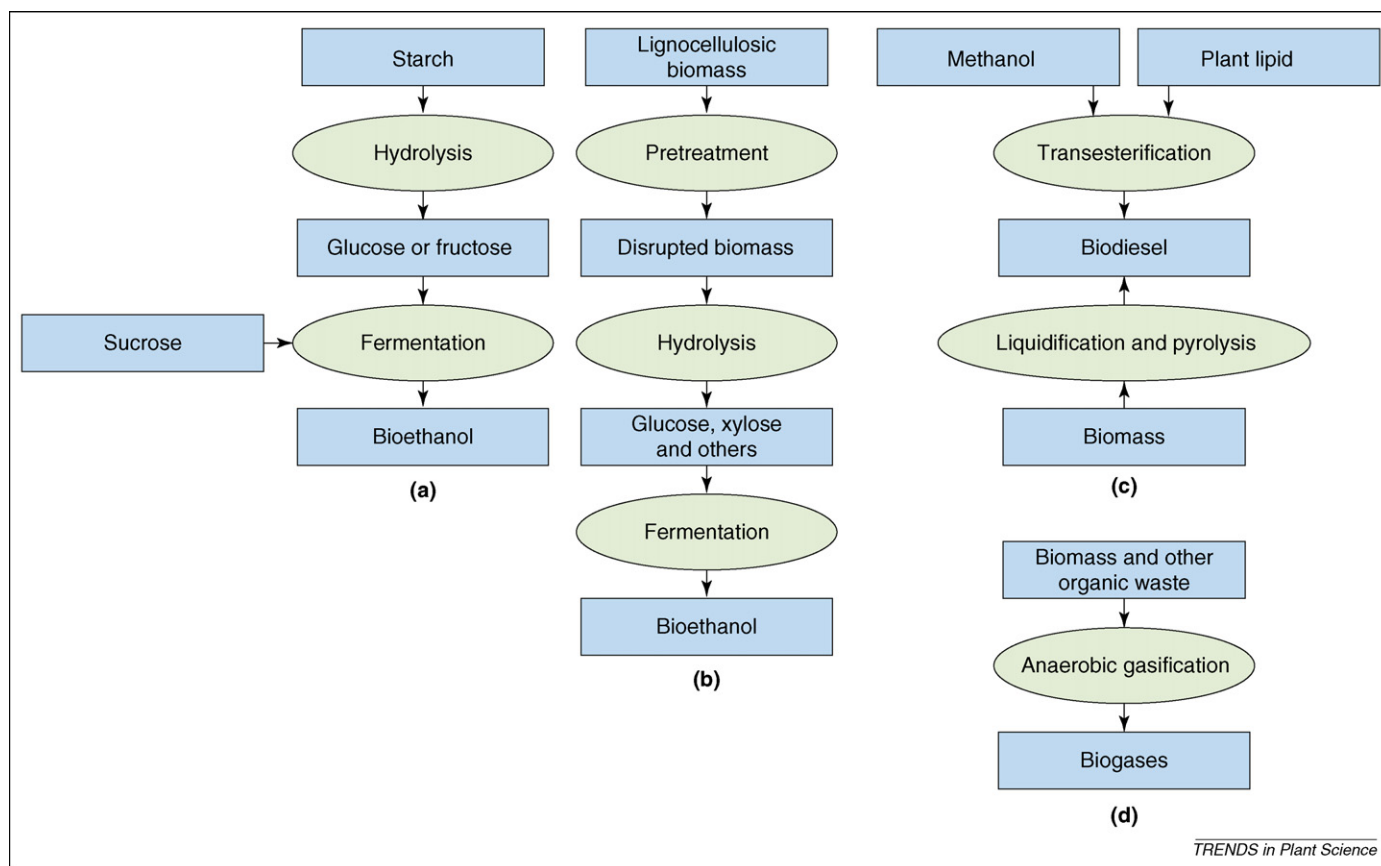
**Net energy ratio (NER):** an alternative measure of energy gain consisting of the ratio of the energy output and the energy input for biomass production and processing.

**Pretreatment:** an initial physical or chemical treatment to disassemble cell wall components, typically involving factors such as high temperature and extreme pH.

**Recalcitrance:** resistance of plant cell walls to hydrolysis for the release of fermentable sugars.

**Saccharification:** the release of products such as cellobiose and glucose from cellulose via chemical hydrolysis or enzymatic reactions.

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**Figure 1.** Processing platforms for producing different biofuels with various feedstocks. **(a)** Starch and sugar-based first generation ethanol. In this platform, starch can be hydrolyzed into monosaccharides, where sugar can be further fermented into ethanol. **(b)** Lignocellulosic ethanol. Lignocellulosic ethanol production involves pretreatment of biomass material, hydrolysis for monosaccharide production, and fermentation to produce ethanol. Lignocellulosic ethanol is believed to be the major component of the second generation of bioenergy. **(c)** Biodiesel. Biodiesel production often involves the transesterification of fatty acids. **(d)** Biomass gasification. Biomass can be used to produce methanol, carbon monoxide, hydrogen or other gases through a process of gasification.

Even though the production of ethanol from starch represents the most convenient and technically advanced option for bioenergy in the USA, it would result in severe competition between energy and food supplies, which is probably not sustainable in the long term given that the net energy and carbon dioxide balance of the platform is not favorable (Table 1) [7,8]. Therefore, in temperate regions, biofuel (ethanol for now) production from lignocellulosic biomass represents the best choice if key tech-

nical hurdles can be scaled. Lignocellulosic feedstock can be acquired from either dedicated biomass crops or forestry and agricultural residuals [5,9–13].

The key obstacle for transitioning from starch-based to lignocellulosic biofuels is the complicated structure of the cell wall, which is, by nature, resistant to breakdown – the recalcitrance problem. Current processes for lignocellulosic biomass include pretreatment, saccharification (hydrolysis), and fermentation (Figure 1b) [14]. Improvement or

**Table 1. Comparison of different platforms and bioenergy crops**

Platforms <sup>a</sup>	Feedstock <sup>a</sup>	NEB <sup>b</sup> GJ/ha/yr	NER <sup>b</sup>	CO <sub>2</sub> balance	Annual feedstock	Estab- lishment	Germ- plasm	Agricul. practice <sup>d</sup>	Ecological benefits	Refs
Ethanol from starch or sucrose	Maize	10–80	1.5–3.0	Positive	Yes	+++ <sup>c</sup>	+++	+++	+	[4,28,30,31]
	Sugarcane	55–80	3.0–4.0	Positive	No	+++	+++	+++	+	[4,28,30,31]
	Sugar beet	40–100	2.5–3.5	Positive	Yes	+++	++	+++	+	[28]
	Sweet sorghum	85–300	5–10	Positive	Yes	+++	++	++	++	[28,92]
Ethanol from lignocellulosic feedstocks	Miscanthus	250–550	15–70	Possibly negative	Yes/No	+	+	+	+++	[28]
	Switchgrass	150–500	10–50	Possibly negative	No	+	+	+	+++	[4,28,30,31,93]
	Poplar	150–250	10–20	Possibly negative	No	+	++	++	+++	[24,30]
Biodiesel	Soybean	–20–10	0.2–0.6	Positive	Yes	++	+++	+++	+	[28]
	Canola	–5–2	0.7–1.0	Positive	Yes	+++	+++	+++	+	[28]
	Sunflower	–10–0	0.3–0.9	Positive	Yes	+++	++	+++	+	[28]

<sup>a</sup>Multiple platforms and crops are compared in a synthesis integrating information from multiple studies.

<sup>b</sup>Abbreviations: NEB, energy balance; NER, net energy ratio, which is the ratio of output to input energy needed to produce a fuel from a feedstock.

<sup>c</sup>Favorable features are indicated by + symbols, with +++ being the most favorable.

<sup>d</sup>Agricul. Practice, agricultural practice: how advanced is the current status of farming, harvesting, and processing.

replacement of these processes is crucial for increasing efficiency and for decreasing biofuel production costs. Obviating pretreatment along with simultaneous saccharification and fermentation are two important factors that would decrease the cost of lignocellulosic ethanol production [14]. Technology breakthroughs are badly needed.

#### *Biodiesel production processes*

Biodiesel is a biofuel requiring far simpler processing technology compared with that required for ethanol. Biodiesel is a mixture of diesel fuel with oils from plant seeds, algae or other biological sources such as animal renderings that have been transesterified for removal of glycerol [15]. A variety of plant species are currently used for biodiesel production including soybean, rapeseed and canola, sunflower and palm [15]. After oil is extracted from plant organs it is transesterified, leading to biodiesel methyl or ethyl esters as products (Figure 1c). Other potential choices for biodiesel include using terpenoid products from *Copaiifera* species ('diesel tree') as biodiesel directly, or engineering the plant terpenoid pathway to produce large amounts of sesquiterpenes and diterpenes. As an alternative to diesel fuel, biodiesel already has a niche in the current transportation fuel system and is widely used, but production is relatively low. The sources and forms of biodiesel are diverse, and it is important to consider the different environmental and economic factors that apply in the production of different types of biodiesel [15]. For example, feedstocks are as diverse as soybean and cooking grease waste. Processing feedstock to fuel is relatively simple, leading to widespread production but of variable quality.

#### *Biogas production process*

A third modern choice for bioenergy is biogas, which is produced from a variety of organic wastes, including plant straw, through gasification (Figure 1d). Biogases include methane, hydrogen and carbon monoxide. Gasification using current technologies has a low net energy balance and its usefulness therefore might be limited [16,17]. Besides the traditional biogases, hydrogen production by green algae and microbes has been proposed as a potential source for a third generation biofuel [5]. Unlike hydrogen production from other biomass sources, algae-based hydrogen production uses a biological water-splitting reaction, in which hydrogenase uses the photosynthetic electron transport chain to reduce protons for hydrogen production [18]. Hydrogenase engineering for increased oxygen tolerance and systems biology research of genes and pathways involved in hydrogen production are needed to realize the potential of this platform [18].

#### **Choices of crops and feedstocks**

Bioenergy crops can be classified into the following four groups: traditional cereal crops, traditional sugar-producing crops, dedicated lignocellulosic biomass feedstocks, and oilseed crops for biodiesel.

#### *Traditional cereal crops*

Cereal crops are a major source for starch-based ethanol production. Maize (*Zea mays*) is an important food and feed crop, used as processed food, oil, fodder, a vegetable and

byproducts. Maize can be used as a bioenergy crop in two ways: the starch in seeds can be used to produce ethanol, and the crop residuals (termed stover) could potentially be used to produce lignocellulosic ethanol. Sorghum (*Sorghum bicolor*) is the fifth most cultivated cereal crop in the world and is grown for grain, forage, sugar and fiber. Sorghum could also be used for bioenergy in several ways. Both the starch in the grain and the sugar could be feedstock for ethanol fermentation using current technology platforms, and crop residuals could be useful for lignocellulosic ethanol production. Two features make sorghum a particularly attractive bioenergy crop. First, there would not be strong competition between the use of land for food or for energy because the seeds can be used for food and feed and the stems could be optimized for different platforms of ethanol production, which is particularly important for heavily populated developing countries such as China. Second, sorghum is drought and heat tolerant, which would enable the usage of marginal land that is not suitable for the cultivation of many other crops [19]. Besides sorghum and maize, the residuals of other crops such as wheat and rice are also expected to be useful for lignocellulosic ethanol production.

#### **Sugar-producing plants**

Sugar can be used for direct fermentation of ethanol. Sugarcane (*Saccharum officinarum*) and sugar beet (*Beta vulgaris*) are the major sugar-producing plants. Sugarcane is adapted to warm temperate to tropical areas, whereas sugar beet is grown in temperate areas. Therefore the two sugar crops occupy different geographical niches. Brazil is a successful example of a country that has reduced its gasoline usage by producing bioenergy. The Brazilian national ethanol program, which is based solely on sugarcane, produces 4.2 billion gallons of ethanol a year [8], although the resultant ecological and environmental effects are still debatable [20,21]. Most ethanol production using sugar beet takes place in Europe; however, using sugar beet to produce ethanol could potentially increase soil erosion and lower the net energy balance. Other sugar-producing crops include energy cane, improved cultivars of sugarcane and varieties of sweet sorghum. All the above crops are annuals, with the exception of sugarcane. Perennials are more desirable than annuals as bioenergy feedstocks because they do not need to be reseeded each growing season and therefore cultivation costs are lower.

#### **Dedicated bioenergy feedstocks**

Perennial bioenergy feedstocks are important sources of lignocellulosic biomass production. Switchgrass (*Panicum virgatum*) has been proposed as the major perennial feedstock in the USA because it is widely adapted, has high biomass production, high C-4 photosynthetic efficiency, and efficient use of water and nitrogen. Switchgrass yield is around 10 to 25 Mg/ha/yr depending on latitude, nutrition and other factors. Hybrid Miscanthus, including *Miscanthus × giganteus*, is another highly favored biomass feedstock, mainly in Europe. However, *Miscanthus × giganteus* is a sterile triploid clone that requires vegetative propagation. Similar to switchgrass, *Mis-*

*canthus* × *giganteus* is also a C-4 perennial plant conferring most of the advantages of switchgrass. *Miscanthus* shows greater cold tolerance and hence might perform better at higher latitudes. The yield of *Miscanthus* × *giganteus* has been reported to be between 7 and 38 Mg/ha/yr and potentially has better nitrogen usage than switchgrass [22,23].

Another group of dedicated bioenergy feedstocks is woody plants, including hybrid poplar, willow and pines. Hybrid poplar is considered a model woody biomass feedstock because of its broad adaptation, available genome sequence and transformation techniques, and fast growth. The biomass accumulation of hybrid poplar is reported to be between ~7 to 20 Mg/ha/yr depending on the nutrition and environmental conditions [24–26]. From the perspective of biomass production, switchgrass and hybrid *Miscanthus* seem to have the potential to produce more biomass compared with that produced by poplar. Given that a short rotation for trees is five years, there is a time lag before poplars can be harvested, and then, only the wood is harvested. Woody biomass does have a storage advantage over herbaceous feedstocks. However, geography, land-use patterns, agronomy, economics and biology are likely to result in multiple feedstock use. Because of the advantages of perennial feedstock, efforts have been put into developing perennial bioenergy feedstocks via breeding [27].

### Plants for biodiesel

In temperate areas, annual oilseeds such as soybean (*Glycine max*), canola (*Brassica napus*), and sunflower (*Helianthus annuus*) have all been used as biodiesel feedstocks. Palm oil (Arecaceae) trees have been successfully used as biodiesel plants in the tropics. If we consider potential biodiesel feedstocks for temperate use, the transportation costs for palm oil would be prohibitively expensive for export and would have a positive net energy balance. In the case of soybean, canola and sunflower, the energy output from grain was estimated to be ~10 to 40 GJ/ha, which is considerably lower than the ~200–500 GJ/ha energy gain from lignocellulosic biomass [28]. Hence, we might conclude that lignocellulosic biomass will have a greater demand than biodiesel feedstocks.

There are other candidates for bioenergy feedstocks that are too numerous to detail in this review. Alternative bioenergy plants include additional crops (e.g. sweet sorghum), *Camelina*, grasses (e.g. big bluestem), trees (e.g. willow), and even algae. Potentially, green algae could be used for hydrogen production, oil production for biodiesel platforms, and even biomass production for a bioethanol platform, depending on the biotechnology breakthroughs. Hydrogen is believed to be an important component of the third generation of bioenergy and can be adapted as different energy sources. Many factors determine the choice of bioenergy crops; these are summarized in Table 1 and discussed further in the next section.

### Bioenergy: environmental, ecological and economic considerations

#### Net energy balance of different platforms

Net energy balance (NEB) is an important concept in choosing a bioenergy platform because only a high

positive NEB can be considered as economically and environmentally sustainable. This is particularly important when considering which crops and conversion processes might be worthy of substantial biotechnology investment. Even though the economics of corn starch-based ethanol and biodiesel production is currently competitive with gasoline, their NEB is fairly low or even negative, in contrast to the favorable NEB of lignocellulosics, as shown in Table 1 [10,28,29]. If lignocellulosic biomass can be efficiently converted into ethanol, a NEB of up to 600 GJ/ha/yr is a reasonable expectation, which would provide the highest NEB of all first or second generation platforms. Recent efforts to build biorefineries for lignocellulosic biomass processing are the first step to fulfilling such potential; however, both low recalcitrance feedstocks and new biocatalysts to improve the processing efficiency are needed to realize this potential. Among the different bioenergy crops, switchgrass, *Miscanthus*, and sorghum could potentially produce the highest NEB [28,30,31].

#### Environmental and ecological benefits of different platforms

Different bioenergy platforms have different pros and cons from an ecological and agricultural perspective (Table 1) [4]. The near-term economic advantages of ethanol production from maize and biodiesel production from soybean are often counter-balanced by the detrimental effects of agricultural practices on the environment. By contrast, perennial feedstocks such as switchgrass can help to decrease soil erosion, improve water quality, and protect natural diversity [4,29,32–34]. Perennial biomass crops also complement food-based and feed-based agriculture instead of competing with it.

#### Global climate change and bioenergy choices

Biomass crops and bioenergy production as an offset to fossil fuel have the potential to ameliorate global warming. Not only does the offset mean that less 'old' carbon is released into the atmosphere, but the underground biomass of perennial biomass crops also acts as a carbon sink. For example, the capacity of *Miscanthus* × *giganteus* to fix carbon dioxide is estimated to be 5.2 to 7.2 t C/ha/yr, which results in a negative carbon balance where more carbon dioxide is fixed than emitted [27]. In a recent study of maize, switchgrass, soybean, alfalfa, hybrid poplar and reed canarygrass (*Phalaris arundinacea*), only poplar and switchgrass had a negative carbon balance (carbon fixation of ~2 t C/ha/yr) [35]. However, two recent publications indicate that first generation ethanol platforms actually have far higher carbon dioxide emissions compared with that released from fossil fuels [20,21]. According to one of the studies, even the best lignocellulosic ethanol is predicted to have a positive carbon balance [20]. These contradictory estimates are a product of the different methods and models used to assess carbon release and fixation. Despite the differences, there is a consensus that second generation ethanol production using lignocellulosic platforms should lead to a lower carbon balance as compared with first generation platforms. Enhancing the ability of perennial feedstocks to



increase their below-ground biomass accumulation will be important for increased carbon storage [8].

#### *Economic considerations*

Current biofuels were marginally profitable before recent petroleum prices spiked. Ethanol production from maize was US\$0.48 per gasoline energy equivalent liter (EEL) in 2005, when the price of gasoline was US\$0.46 per liter [29]. However, the recent spike in the price of global crude oil has made maize-based ethanol production very profitable. The situation is similar for biodiesel production: soybean-based biodiesel production was US\$0.55 per diesel EEL in 2005 [29]. Despite these potential profit margins, traditional crop-based biofuel production has led to direct competition between food and energy and, as a result, the recent crude oil price increase has already led to the global inflation of the cost of food, feed and associated products. There is an imminent need to move to lignocellulosic biomass-based platforms. However, lignocellulosic ethanol production using the current platforms is not profitable [36]. The lignocellulosic ethanol price is still as high as US\$0.70 to US\$1.0 per EEL. However, as processing technologies mature and biomass crops are modified for higher yield and lower recalcitrance, the cost of lignocellulosic-based ethanol production is expected to decrease to rival maize grain platforms. Indeed, based on break-even price yield, recent analysis has already indicated that switchgrass is a more profitable crop than traditional crops such as sorghum and maize [10]. The competitiveness of lignocellulosic ethanol as a sustainable energy supply in the USA will therefore heavily depend on biotechnology breakthroughs to reduce cost and improve processing efficiency.

#### **Plant biotechnology solutions for bioenergy**

Novel enabling biotechnologies are crucial for reducing the costs of bioenergy production, particularly of lignocellulosic ethanol. The key issues include rapid domestication, overcoming recalcitrance, efficient breakdown of cellulose, and increasing biomass and lipid production for ethanol and biodiesel, respectively [37]. Although aspects of these important areas will be discussed individually below, it is important to solve these problems in concert.

#### *Modification of lignin biosynthesis*

Lignin might be the most crucial molecule in need of modification for lignocellulosic feedstocks. It has been established that reducing lignin biosynthesis can lead to lower recalcitrance and higher saccharification efficiency [13,38–54]. Recent studies have indicated two important aspects for lignin modification. First, both lignin content and composition are important. Although it is codependent on efficient processes to fractionate lignin, a more uniform lignin structure might facilitate more efficient cell-wall degradation for fuel production (John Ralph, personal communication). Second, the pretreatment of biomass might even be rendered unnecessary if lignin content falls below a critical threshold, which would enhance downstream enzymatic saccharification and fermentation steps for improved efficiency [55]. Therefore, switchgrass, Miscanthus or poplar feedstocks with modified lignin can improve the efficiency of biomass conversion into fermentable

sugars [55]. Lignin biosynthesis in monocot species should be studied further so as to be able to modify lignin biosynthesis intelligently in perennial grass feedstocks.

#### *Preprocessing in planta: expression of cellulases and cellulosomes*

Plant cell walls can be degraded by individual cellulases or in concert by cellulosomes, which consist of a suite of enzymes. *In planta* expression of cellulases and cellulosomes could potentially reduce the cost of enzymatic saccharification of lignocellulosic biomass at the biorefinery by providing the enzymes needed for cell wall degradation. The effectiveness of *in planta* expression of free cellulases or cellulosomes is still controversial because digestion is complicated by the subcellular localization of enzyme(s), glycosylation of extracellular proteins, effective enzyme combinations, and the requirement for inducible expression to avoid premature cell wall digestion.

Regardless of the challenges, preliminary research has shown the successful apoplastic expression of active *Acidothermus cellulolyticus* cellulase E1 in maize and tobacco [56,57]. Moreover, recent research has indicated that maize plants showing *in planta* expression of cellulase had higher biomass conversion efficiency [58]. Researchers have indicated that no detrimental effects were found by apoplast targeting of E1 and that cellulase activity aided biomass conversion when plant material was milled [56,59]. These differences result from the different subcellular localization and activation mechanisms of the enzymes. For example, heat-activated cellulase enzymes such as E1 have no detrimental effects to plants growing in typical ambient temperatures [55,56].

Further study is necessary to determine different strategies for *in planta* cell wall digestion enzyme expression, with high-throughput approaches for optimized subcellular localization and different combinations of enzymes. In addition, fundamental research still needs to be performed to modify cellulases for improved catalytic efficiency, thermal stability, performance under extreme conditions, along with protein modifications designed to lead to reduced apoplastic glycosylation. Many cell-wall degrading bacteria use cellulosomes, a suite of enzymes for cell wall hydrolysis [60]. Cellulosomes have not yet been expressed in transgenic plants, but the correct assemblage of cellulosome components in the plant apoplast potentially have the promise of decreasing recalcitrance and facilitating the post-harvest hydrolysis of cellulose, which in turn might aid simultaneous saccharification and fermentation.

#### *Abiotic stress resistance*

Suboptimal water and other abiotic stresses are limiting factors for biomass production; stress tolerance traits are therefore important to enable feedstock to be produced on marginal or sub-marginal lands not favorable for food crops. Drought-, metal-, salt-, cold- and heat-stress all induce some similar responses in plants, yet each of these stresses will induce a different set of genes [61]. The upstream pathways for salt and drought stresses have been well-characterized in *Arabidopsis thaliana* [62–64], but until recently have led to only limited success in translational research to produce field crop abiotic stress

tolerance. Improved cold and drought tolerance has been reported in tobacco and potato by transformation with the gene encoding DREB1A (dehydration response element B1A), which is driven by a promoter of a stress-responsive water channel, RD29A [65,66]. Rice plants with induced expression of a NAC (for NAM, ATAF and CUC)-type transcriptional factor, OsNAC6, have been shown to enhance tolerance to both high salinity and plant pathogens [67].

More research is required to understand the effects of abiotic stresses on bioenergy crops from two different perspectives. First, genetic variation among different cultivars should be explored both in the laboratory and in field studies, which will guide breeding and genetic engineering for feedstock improvements. Indeed, switchgrass shows large phenotypic variation for water and cold-stress tolerance even within cultivars [11,68,69]; the different cultivars might be incorporated in breeding programs for better-adapted feedstocks. Second, basic science from model species needs to be translated into field crop improvement, and many key stress-response genes identified in model species should be explored for the genetic modification of bioenergy feedstocks such as switchgrass and poplar.

#### *Increasing biomass production and yield*

The importance of altering plant growth and development to increase the biomass production for bioenergy cannot be over-emphasized. Given that most lignocellulosic biomass crop candidates are relatively undomesticated, rapid progress should be attainable. First of all, the molecular mechanisms controlling plant architecture need to be better understood. Current knowledge in the field can be translated into developing bioenergy feedstocks with desirable architectural features such as dwarf stature and erect leaves. It has been shown that these features can be achieved by modifying biosynthesis or signal transduction for key plant growth hormones including GA (gibberellic acid), IAA (indole-3-acetic acid) and brassinosteroids [70–74]. Biotechnology could make rapid improvements in bioenergy feedstocks using genomics-guided improvements. For example, GA pathway genes such as *Δgai* (gibberellic acid-insensitive) could be introduced into switchgrass to dwarf the plants, which should produce a crop with an increased annual biomass that is easier to harvest [75]. In addition, dwarfing might also help to change the lignin content of the overall biomass. Following dwarfing, biomass allocation should shift to the leaves. The leaves of switchgrass have been shown to contain a lower proportion of lignin than that found in stems [76]; dwarfing would increase the cellulosic content needed as feed, or for saccharification and fermentation needed for ethanol production. One of the major goals of poplar domestication is to produce dwarf Trees – pathways that are controlled by auxin, GA and brassinosteroids could potentially be altered to achieve this goal [70–74].

Second, developmental programming of feedstock needs to be altered to increase biomass production. For example, delaying the onset of flowering has been reported to result in increased biomass [77]. Third, biomass production can also be increased by the genetic modification of cell wall

biosynthesis and modification enzymes: the overexpression of cellulose synthase in poplar has led to higher lignocellulosic biomass biosynthesis [78]. Overall, the production of biomass can be further increased with the engineering of plant hormone response genes or genes involved in developmental processes [70–74,77,79,80].

#### *Male sterility and biocontainment*

Male sterility is another desirable feature for feedstock development to prevent transgene escape from genetically modified feedstock [1]. Induced male sterility is one approach to limit transgene flow. Male sterility can be induced in plants by either knocking out the expression of genes important in pollen development or pollen-specific silencing of major metabolic genes [81–83]. Another approach to prevent transgene flow is the excision of the transgene in pollen through pollen-specific recombinase activity [84,85]. Most of the proposed bioenergy crops such as switchgrass have wild relatives, and transgene flow is expected to be a major issue limiting the application of genetic engineering in these species. Preventing transgene flow is therefore an important issue for feedstock improvement by genetic modification.

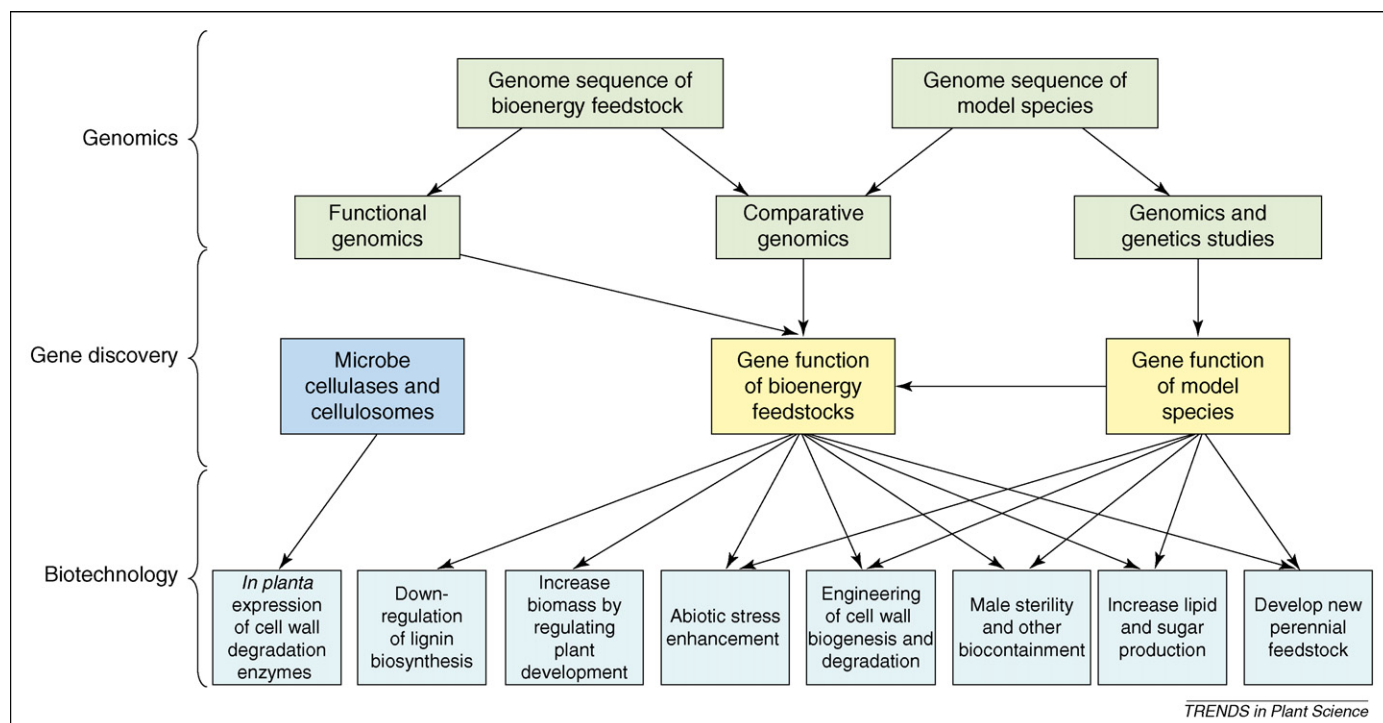
#### *Metabolic engineering*

Metabolic engineering will play an important role in improving biodiesel, biomass and sugar production. The future of biodiesel will largely depend on metabolic engineering to improve oil content and composition in seeds [15,86–89]. Previous oilseed research has focused mainly on changing fatty acid profiles, particularly for nutritional purposes [87,89]. Recent efforts have also led to an increase in lipid production via induced expression of key exogenous lipid biosynthesis genes [88]. Metabolic engineering can also help to increase the production of sugar and starch for ethanol production using current platforms [90]. For example, recent research has indicated that the overexpression of a bacterial sucrose isomerase in vacuoles could double the sucrose yield for sugarcane [90]. Metabolic engineering will also become an important approach for increasing non-fuel bioproducts, and advanced bioproducts might be the greatest long-term benefit of the current biofuels research spike. Although it is possible that some alternative, non-biobased, fuel could ultimately replace petroleum, plastics and other bioproducts will require new feedstocks in the absence of petroleum feedstocks.

Overall, plant biotechnology will play a central role in the next generation of bioenergy options to produce lignocellulosic feedstocks with higher yield, better water-use efficiency, greater net energy gain, lower recalcitrance, enhanced abiotic stress tolerance, and improved ecological benefits, such as better carbon fixation and water and soil conservation.

#### **Fueling the future**

The future of bioenergy will depend on breakthrough technologies. However, the importance of basic research on pathways and genes involved in cell wall biosynthesis, plant development, and metabolite production should not be ignored. Translational systems biology is needed for biofuel applications (Figure 2) [91]. The use of 'omics'



**Figure 2.** Genomics-based biotechnology research for bioenergy. Plant biotechnology research greatly depends on the availability of genome information. The process from genomics to gene discovery to biotechnology is shown. Basically, the genomics level information (green boxes) will lead to gene discovery (yellow boxes), which in turn be translated into biotechnology for feedstock improvement (light-blue boxes). In addition to plant biology research, studies using microorganisms (deep-blue box) are also important.

techniques should be helpful to study the genes, proteins and metabolites from different tissues and at different developmental stages to correlate the features and structures of cell walls with genes of interest for guiding further gene discovery and biotechnology-based feedstock improvements.

Furthermore, bioenergy is not, and should not be, limited to higher plants, although higher plants are likely to provide the most important feedstock for first and second generations of biofuels. Studies of microbes that have the capacity to digest plant cell walls will also be important components of bioenergy research. In addition, green algae should be considered as a potential feedstock choice because of their fast growth. Biofuel cells might also be an option if more mature technologies become available through engineering breakthroughs. Overall, bioenergy research is emerging as a field full of opportunities to re-shape the energy supply of human society.

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