F1000Reports
BIOLOGY

Published: 02 May 2012

Development of feedstocks for cellulosic biofuels

Heather Youngs* and Chris Somerville

Address: Energy Biosciences Institute, University of California Berkeley, Berkeley CA 94720, USA

* Corresponding author: Heather Youngs (hlyoungs@berkeley.edu)

F1000 Biology Reports 2012, **4**:10 (doi:10.3410/B4-10)

Abstract

The inclusion of cellulosic ethanol in the Energy Independence and Security Act (EISA) of 2007 and the revised Renewable Fuel Standard (RFS2) has spurred development of the first commercial scale cellulosic ethanol biorefineries. These efforts have also revived interest in the development of dedicated energy crops selected for biomass productivity and for properties that facilitate conversion of biomass to liquid fuels. While many aspects of developing these feedstocks are compatible with current agricultural activities, improving biomass productivity may provide opportunities to expand the potential for biofuel production beyond the classical research objectives associated with improving traditional food and feed crops.

Introduction

It is widely believed that anthropogenic loading of greenhouse gasses, such as CO_2, N_2O and methane, into the atmosphere is causing detrimental climate change. The majority of CO_2 emissions arise from burning fossil fuels, which supply about 85% of human energy consumption globally. In response to this, there are currently many low-carbon methods to generate electricity, including wind, geothermal, hydroelectric, and solar approaches. When considering the likely contribution of these approaches, it is useful to remember that the Earth receives approximately 7000 times as much energy from the sun as all human energy uses [1]. Energy from the sun can be utilized in the following three ways: via photovoltaic conversion to electricity, by using mirrors to heat liquids that power sterling engines to produce electricity, or by harvesting plant biomass that can be burned as solid or liquid fuels. At present, none of these approaches can provide for all our energy needs. Thus, it is essential to approach renewable energy production through a basket of complementary technologies rather than to rely on a single technology. Generating biomass is currently the most cost-effective route to produce renewable liquid fuels. At present, in the USA, biomass provides about 40 times as much energy as photovoltaics [2] and represents 78% of the total renewable energy worldwide [3].

Liquid biofuels are currently made almost entirely from sugar, starch, or fats and oils derived from plants that are also used for food and feed. Appropriately, there is concern that the use of food and feed crops for fuel may not be sustainable in the face of expanding demand for food, feed and fiber. However, there is a long-term opportunity to produce fuels from non-edible lignocellulosic biomass from plants. In this brief review, we have summarized some of the issues associated with development of feedstocks for cellulosic transportation fuels and have attempted to outline some of the scientific questions in plant biology that are related specifically to this topic. Many other reviews of this subject and related matters have appeared [4-9], some include a particularly dynamic topic that is beyond the scope of this review – estimates of the impact of land use for biofuels on other uses of land ('consequential' or 'indirect' land use change). In general, it is apparent that some land is available for production of biofuels without significant effects on food production or on ecosystem services [10-12]. A recent study estimated that more than 600 million hectares of land worldwide have fallen out of agricultural production, mostly in the last 100 years [11]. Some of this area appears suitable for the production of perennial grasses or other types of energy crops, but additional research is necessary to categorize the land with respect to the potential for various types of energy crops. Our view is that biofuels can probably be produced on a large enough scale to meet demand for about 30% of all liquid transportation fuels [10]. Improvements in energy efficiency could significantly increase the percentage of transportation fuels produced from biomass.

First generation biofuels

At present, liquid biofuels are of two main types: ethanol and biodiesel (fatty acid methyl esters of lipids). Ethanol is primarily made by using yeast to ferment sugar extracted from sugarcane and sugarbeet, or from the depolymerized starch of corn, wheat or cassava. Some countries are already using this technology to provide significant levels of fuel. In 2009, in Brazil, production for fuel on about 4.6 million hectares resulted in about 27 billion liters of ethanol plus two gigawatts of net electricity from combustion of bagasse (the residual lignocellulosic material of sugarcane stems left after processing for sugar) [10,13]. The introduction of flex-fuel vehicles into the Brazilian light-duty fleet allows high-blending (all gasoline in Brazil contains 25% ethanol) as well as mixing of ethanol and gasoline by the consumer at the pump. In 2008, ethanol replaced about 40% of the gasoline used in Brazil [14]. The Brazilian government recently announced that expansion of the sugarcane crop would be limited to 63.5 million hectares [15]. This land could be made available without the clearing of natural ecosystems by a slight increase in the low stocking density of cattle ranching, which currently occupies an estimated 237 million hectares [14]. Advances in sugarcane breeding and agronomy, in addition to process improvements in ethanol production from sugar and bagasse, are also expected to significantly increase the amount and efficiency of production, while also improving the environmental impact of sugarcane production [16].

In 2011, about 49 billion liters of ethanol were produced by fermentation of corn-derived sugars from 38% of the 32.2 million hectares of land used to grow corn for grain in the US [17]. Most of the increase in use of corn for ethanol during the period 2000-2009 was provided by an increase in yield of the US corn crop during that period [17]. Approximately 35% of the mass of corn kernels is recovered from the ethanol production process as a high-protein residue, called dried distiller grains, which has high nutritional value and is used as cattle feed. Although expansion of corn has displaced some soy production, the feed value of the dried distiller grains from a hectare of corn processed for ethanol is similar to the feed value of soy protein obtained from a hectare of soybean. Use of corn grain for ethanol production also creates large amounts of stover, leftover leaves and stalks similar to the bagasse in sugarcane production. Unlike sugarcane bagasse, which is combusted for process heat and electricity to drive the process, corn stover is typically unused and left in the field. Recent studies indicated that the net energy return for corn ethanol could be almost doubled by using stover for production of process heat and electricity [18-19].

Biodiesel is produced primarily from triacylglycerol obtained from soy, canola and other oilseeds or from the mesocarp of palm fruits. Tallow, lard and used cooking oil can also be converted to biodiesel but are available in relatively small amounts. The conversion is conveniently accomplished by trans-esterifying triacylglycerols with methanol to produce fatty acid methyl esters and glycerol. Fatty acid methyl esters can be used directly in diesel engines, whereas ethanol cannot. Because temperate oilseeds have much lower biofuel yields than corn, economic incentives favor ethanol production wherever corn can be grown.

Advanced biofuels

Biofuels made from feedstocks other than starch, sugar or lipids are generally referred to as advanced biofuels. The largest source of feedstock is lignocellulose, the cell walls that comprise the bodies of higher plants. There are several different technologies for conversion of lignocellulose to fuels, and the choice of technology can have substantial implications for the environmental and agronomic aspects of biofuel production [6,8,20]. We consider here only biochemical conversion technologies.

The potential attractiveness of bioconversion technologies for liquid fuel production is related to the idea that they may have lower capital costs than thermal conversion methods at scales that do not require long distance transport of biomass (e.g. ~35-100 million gallons per year). The key steps in bioconversion of lignocellulose to fuels are size reduction through grinding, pretreatment, hydrolysis and fuel production [20]. The role of pretreatment methods is to increase the porosity of biomass particles and to increase the accessibility of cellulose and other polysaccharides to enzymes [21]. One of the most developed methods involves heating in dilute acid such as 1% H_2SO_4. Treatment for a little as two minutes at 180°C in 1% H_2SO_4 can result in depolymerization of as much as 90% of the hemicelluloses (i.e. xylan) [22]. Since a substantial amount of xylan is thought to be hydrogen-bonded to the surface of cellulose microfibrils, the acid pretreatment presumably exposes the cellulose microfibrils to some extent, both by hydrolysis of xylan and also by releasing lignin from indirect association with cellulose via linkage to xylan. Other methods, such as ammonia fiber expansion cause similar effects [21].

Following pretreatment, solubilized sugars are separated from solids, which are subjected to further hydrolysis catalyzed by enzymes that can collectively hydrolyze cellulose and residual hemicellulose to free sugars [23]. However, relatively large amounts of enzyme (e.g. ~25 kg/ton of cellulose) are reportedly required to release most of the sugars from biomass at rates compatible with the high-throughput production of fuel [20]. Aside from capital costs, the requirement for unusually large amounts of enzymes appears to be the single largest cost in the production of cellulosic fuels from non-thermal routes. Not surprisingly, this has led to a search for more active glycosyl hydrolases from incompletely explored sources, such as termites and cow rumen [24].

The principal components of plant cell wall polysaccharides from most higher plants are glucose and xylose. In some species (e.g. conifers and seaweeds) mannose is also abundant. Thus, a minimum capability of an industrial biofuel-producing microorganism is the ability to convert these sugars to liquid fuel components, such as ethanol or other alcohols, alkanes or terpenes. Although the industrially adapted strains of yeast that are used for ethanol production cannot use xylose, strains of yeast with this capability have been developed through genetic engineering [25-26], and many naturally occurring yeast and bacteria have this capability.

In addition to using all sugars, microbial strains must be resistant to the compounds produced or released during biomass degradation [20,27]. Because it is desirable to have concentrated sugar solutions in order to minimize volume and maximize concentration of product, the initial concentration of the biomass is very high. This may result in a pre-treated solution or hydrolyzate that also contains relatively high concentrations of toxic compounds. Thus, for instance, acid pretreatment of biomass produces dehydrated sugars, such as furfural and hydroxymethyl-furfural that are toxic [27-28]. In addition, many polysaccharides are acetylated and some pretreatments release acetic acid, which can be toxic at high concentrations. These compounds cause cell stress, especially in conjunction with high sugar concentrations, limiting fermentation efficiency. The biological function of acetylation is unknown, so it is not known to what degree this property of cell walls can be modified without deleterious effects [29-30]. Finally, some potential biomass crops contain secondary metabolites that are toxic to microorganisms [31-32]. Identification and elimination of such compounds by genetic methods is a priority for research on plant feedstocks if it can be done without creating pest and pathogen problems.

Biofuel feedstocks

In terms of global grain or seed production, maize is the largest crop, producing about 820 million megagrams of grain and a similar amount of stems and stripped cobs, collectively referred to as stover, that is potentially available for fuel production [10]. Conversion of half of the maize stover in the US to cellulosic ethanol would produce about 13.5 billion gallons of ethanol. However, there is concern that removal of even half the stover would exacerbate loss of soil carbon and erosion and would also require additional inputs of fertilizers to replace lost minerals [33]. Also, the relatively low amount of residues produced per hectare from other crops may impose unacceptably large costs for collection and transportation to refineries [34].

C_3 and C_4 plants fix CO_2 into a compound with three of four carbon atoms, respectively, before entering photosynthesis. Perennial C_4 species, such as sugarcane, energy cane, elephant grass, switchgrass, and Miscanthus, have intrinsically higher light, water and nitrogen use efficiency than C_3 species. Additionally, with perennial C4 plant species reduced tillage and perennial root systems add carbon to the soil and protect against erosion. Seasonality leads to an annual cycle of senescence, whereas perennial grasses, such as Miscanthus, mobilize mineral nutrients and carbohydrates from the stem and leaves to rhizomes at the end of the growing season. Consequently, harvest of biomass from perennial grasses during the winter months has the advantage that it allows relatively high retention of minerals in the rhizomes, reducing or eliminating the need for fertilizers [35]. Additionally, storage of carbohydrates in the rhizomes allows rapid early growth following emergence of new shoots in the spring, contributing to a high biomass yield. In one study at Rothamsted UK, all above-ground biomass was removed each year over a fourteen year period from stands of Miscanthus with no decrease in yield and no response to added nitrogen [36-37]. Side-by-side trials in central Illinois showed that unfertilized *Miscanthus x giganteus* produced 60% more biomass than a well-fertilized, highly productive maize crop and, across the state, winter harvestable yields averaged 30 megagrams/hectare/year [35,38]. Mechanistic models developed to project yields based on the extensive trials conducted in Europe, when applied to the USA, suggest that many locations east of the Mississippi could support average annual yields of over 30 megagrams/hectare, with significant areas exceeding 40 megagrams/hectare [10]. Work on tall perennial grasses native to the American plains, such as switchgrass (*Panicum spp.*), prairie cordgrass (*Spartina spp.*), big bluestem (*Andropogon spp.*) little bluestem (*Schizachyrium spp.*) and others, could produce significant biomass in a variety of biomes throughout the nothern plains and southeastern grasslands [39].

Woody biomass can be harvested sustainably for lumber and paper and may, therefore, provide biofuel feedstock for some regions [40]. By one estimate, the biomass that is harvested annually in the Northern Hemisphere for wood products has an energy content equivalent to approximately 107% of the liquid fuel consumption in the US [41]. Globally, large areas of land formerly used for agriculture have reverted to forest [42], and the continuing trend to electronic media and paper recycling may reduce the demand for pulp woods. This presents an opportunity to reallocate woody biomass for energy. In order to maximize the amount of woody biomass produced per hectare, the best practice appears to be coppice harvesting, in which the plants are cut near ground level after the end of the growing season every three to five years, depending on the species and the growing conditions [43]. A wide variety of trees are amenable to coppicing, although willow (*Salix spp.)* has been the most studied in northern regions and eucalyptus in the south. Coppice trees rapidly regenerate shoots from the rootstock without any intervention. This approach minimizes losses of mineral nutrients, soil erosion and organic carbon emissions and the investment of photosynthate in regrowing the roots.

The amount of land required to produce enough biofuel to have a significant impact on demand depends entirely on the productivity of a given feedstock on a given parcel of land. The productivity is, in turn, governed by a wide variety of physiological factors, including genetic diversity, agronomic practice, and environmental factors, such as soil quality, water availability, and climate [44-45]. Thus, predicting the amount of land required to produce enough biofuel to impact fossil fuel demand can be quite variable [46], even for a single biofuel crop. The use of residual biomass from agricultural, forestry, and municipal activities decreases the amount of land needed for energy crops [9,47]. Likewise, the development of energy crops adapted to be highly productive on lands marginal for other agricultural uses will be needed to reduce the potential impact of biofuel production on crop production. Clearly, decisions regarding land use and feedstock choices will have an impact on how much fossil fuel can be replaced. Growing perennial grasses on the 13 million hectares of land that farmers in the US are paid to keep out of production to support commodity prices, combined with available crop and forest residues, could provide enough fuel to meet 65% of the demand for gasoline in the US [9]. The actual availability of land for biofuel production will be determined by politics as societies weigh competing demands for land.

Potential new bioenergy crops

Approximately 18% of the terrestrial surface is semi-arid and prone to drought and, therefore, does not support crop production [10]. Indeed, much of the roughly 600 million hectares of land that has fallen out of agricultural production worldwide is semi-arid [42]. It may be possible to utilize this type of land for bioenergy production by using species with high water-use efficiency and drought resistance [10]. For example, *Agave* spp. utilize a type of photosynthesis called Crassulacean acid metabolism that strongly reduces the amount of water transpired during growth. Thus, *Agave* spp. have a water use efficiency that may be as much as six times greater than that of C_3 species, such as wheat [48]. Several Agave species (native to hot, dry regions) have been cultivated for production of sisal coarse fibers (*Agave sisalana*, *Agave fourcroydes*) or alcoholic beverages (*Agave tequilana*, *Agave salmiana*), so agronomic practices are well established. Some Agaves have been reported to exhibit high harvested biomass yields on semi-arid land when harvested on 5-6 year cycles [49].

Salinization has also become an increasing problem in marginal agricultural lands. The United Nations Food and Agriculture Organization (FAO) estimates that 1-2% of irrigated lands are lost to salinization each year. Salinity also restricts the use of treated wastewater for irrigation. Most crops, such as wheat and rice, are highly salt-sensitive. Thus, salinized soils generally have low productivity and are not utilized for food crop production. Research on salt tolerant species, such as prairie cordgrass [50] and *Eucalyptus spp*., could be useful in bringing these lands back into production as well as improving salt tolerance in other crops. A recent estimate by Wicke and colleagues indicates that growth of salt-tolerant trees on nearly a billion hectares of saline land could produce 5-11% of global primary energy consumption annually [51].

More generally, a challenge for plant biologists is to identify the most highly productive plant species that can be grown on the various types of marginal or abandoned land, to optimize the genetics and production practices, and to evaluate any environmental risks or benefits that may accrue from encouraging the widespread use of such species for energy production. A particularly important topic in this respect is to identify species that are not invasive, or to develop technologies, such as conditional sterility, that can prevent invasive spread.

Improving biomass yield

In order to minimize the amount of land diverted from other purposes to energy production it is essential to maximize the "yield" – the amount of biomass produced per unit of land. Many of the goals of breeding perennial grasses for maximal yield have been described in an excellent review [52]. One of the primary goals is to understand the diversity of biomass traits [53] and the regulation of carbon partitioning and biomass production [54-55]. Identifying and dissecting the complex interaction between stresses, hormones, and signaling pathways on biomass synthesis and composition is underway [56-62].

Unlike crops used for food, where much of the yield gain during the past century was accomplished by minimizing the amount of biomass in the leaves, stalks and roots in favor of grain yield, the opposite strategy is desirable for energy crops. Indeed, prevention of flowering may allow plants to remain in a vegetative growth phase longer than for plants that undergo the transition to flowering, thereby extending the period of biomass accumulation [63]. Additionally, allocation of resources to seed development is relatively inefficient compared with production of additional leaf and stem biomass. Thus, eliminating production of fruit or seeds is likely to increase total biomass and may also be beneficial in preventing invasive propagation. However, in order to propagate energy crops with maximal efficiency, it would be desirable to develop systems that allow production of seeds under controlled conditions, as propagation by other means (e.g. tissue culture, rhizomes, or cuttings) is often more expensive and may require specialized equipment.

One possible strategy for implementing conditional flowering is to regulate the photoperiodic induction of flowering (i.e. in response to the amount of daylight in a 24 hour period), so that it takes place in latitudes where the crop is not normally produced. Thus, for example, seed might be produced in equatorial regions for crops grown at non-equatorial latitudes. Recent progress in understanding photoperiodic induction may allow new approaches to engineering this trait into energy crops [64]. Similarly, progress in developing chemical control of gene expression in plants may allow the development of crops that flower only when treated with an inducing compound.

Because bioenergy crops are expected to be grown on marginal land that is not suitable for major food crops, it will be desirable to identify varieties with robust environmental stress tolerance. In addition to traits that are of interest for food crops, such as drought tolerance, it may be desirable to develop flooding tolerance, as water is frequently limiting to plant growth. Unusually wet acres are typically difficult to farm with annual row crops but provide an opportunity for maximal biomass production using perennials. Understanding how nutrients are stored and remobilized [65,66] and improving nutrient acquisition [67] are important for maximizing perennial traits.

Selection for cold tolerance during overwintering will probably be important in extending the range of highly productive perennial biomass species, such as sugarcane. In some cases, the tolerance may be achieved by production of interspecific hybrids, such as Miscane – a hybrid between sugarcane and cold-tolerant Miscanthus sp [52,68]. The importance of cold tolerance may extend to tree species, such as the very productive but very cold-sensitive Eucalyptus species, in which engineered cold tolerance has been demonstrated.

One of the most worrisome threats to production of perennial bioenergy crops is loss to pests and pathogens [69]. Recent studies of prospective crops, such as switchgrass and Miscanthus, have confirmed that these species harbor a wide variety of viruses, fungi, insects and nematodes. Because these crops will be replanted at long intervals (e.g > 10 years) it will not be possible to minimize biotic stress by alternating genotypes or species. Thus, pest and pathogen populations could grow. Presumably, the best strategy will be diversification of the plantations to maximize the genetic diversity of relevant tolerance and resistance pathways. Mixed stands of species that mimic natural grasslands could accomplish this and provide habitats for more diverse populations of organisms. Although there has been some research suggesting that natural grasslands have higher productivity than pure stands of grassland species [70], other results suggest that the effect may apply only to very low productivity settings [71]. Even in mixed stands, it will be desirable to maximize genetic variability within each species. The fact that energy crops are likely to be harvested after the end-of-season senescence implies that concerns about uniformity of maturity and stature that have limited the use of "multilines" in breeding of food crops will be relaxed. However, the use of diverse genotypes may trigger problems in managing interplant competition that has been implicated as a factor limiting yield in older varieties of some crops, such as maize and wheat.

Modification of plant architecture may also play an important role in maximizing biomass yield. The existing varieties of energy crops, like Miscanthus and switchgrass, have a tendency to drop their leaves during late-season senescence, thereby significantly reducing yield. Selection for leaf retention could have a major benefit. More generally, plant height, tiller number, leaf density and stem thickness are determinants of biomass productivity [52], and there is often a trade-off in growth habit and architecture between tolerance for high-density planting and efficient photocapture. Altering root architecture may be a good approach as it increases water and nutrient capture efficiency as well as soil carbon [72].

Biomass composition

Most of the mass in the bodies of plants is attributable to the polymers cellulose, hemicelluloses and lignin. Thus, biochemical conversion processes are designed to depolymerize the polysaccharides to sugars that can be fermented to fuel, whereas the lignin is burned to produce heat and electricity to support the overall biomass-to-fuel conversion process. At present, the various conversion processes that are advancing towards commercialization have high capital costs and high operating costs per unit of fuel produced, relative to the price of petroleum-based fuels. Consequently, there is a lot of interest in the possibility of altering the chemical composition or structure of biomass genetically to render the conversion process less expensive.

One focus of research is the effect of variation in lignin content and composition on enzymatic depolymerization of biomass [73]. Because lignin partially occludes the polysaccharides, and because it cannot be readily depolymerized, the presence of lignin directly impedes polysaccharide depolymerization. Additionally, binding to lignin appears to inactivate many of enzymes that are used to depolymerize cell wall polysaccharides. The interplay between lignin and cellulose has long been recognized. On the plus side, reduction in lignin is often accompanied by increased cellulose and hemi-cellulose deposition [74-76]. However, on the minus side, significant reduction in lignin content is accompanied by growth impairment [77]. This may be, in part, because some of the intermediates in lignin synthesis are precursors to hormones and other important secondary metabolites that may have signaling roles. It was recently observed that reduced lignin was accompanied by increased salicylic acid, presumably induced in response to a signal sensed as cell wall damage [78]. Thus, it is possible that some of the negative effects on growth might be alleviated by decoupling the cell wall integrity-sensing system [79]. A notable recent study in which alfalfa caffeic acid O-methyltransferase was downregulated resulted in plants with slightly reduced lignin, but modified cell wall composition that significantly reduced the severity of pretreatment and the amount of enzymes required to depolymerize polysaccharides [80]. Studies of natural variation in lignin content and composition have indicated that it is possible to significantly improve the digestibility of biomass by selection for certain compositions [81]. This and related work clearly illustrates opportunities to modify lignin with useful effects [82,83]. However, much remains to be done to understand the mechanistic basis for the effects. Also, it remains to be seen if the conditions used to test depolymerization in laboratory studies of a species that is not a suitable energy crop will be relevant to the industrial conditions that are under development.

If it were possible to separate lignin from polysaccharides before depolymerization of biomass, the problems caused by lignin might be significantly ameliorated. However, none of the pretreatment processes remove all of the lignin [84], possibly because, in herbaceous species, lignin is covalently linked to hemicelluloses that are hydrogen bonded to cellulose. Thus, a largely unexplored opportunity might be to reduce or eliminate the enzymes that catalyze cross-links between lignin and hemicelluloses. Perhaps the most creative idea about how to facilitate lignin removal is to introduce new components into lignin that are susceptible to enzymatic or chemical cleavage [83].

Another approach to the modification of biomass composition is to increase the abundance of easily depolymerized polysaccharides. In principle, it may be possible to significantly increase the amount of cellulose, hemicelluloses or other polysaccharides in cell walls, but the mechanisms that control synthesis and deposition of these polymers are not yet known. However, it was recently observed that the corngrass1 (Cg1) gene of maize, which encodes a microRNA, promotes juvenile cell wall composition and starch accumulation in maize, and also when transferred to switchgrass [85]. These effects increased the amount of sugar released by depolymerization (saccharification). It will be important to carry out careful measurements of the effects of such alteration on total yield per acre under a variety of growth conditions in order to know whether such changes have practical utility.

Many polysaccharides are acetylated and, during the processing of biomass, acetate is released and subsequently inhibits metabolism of the microorganisms that convert sugars to fuels. The biological function of acetylation is not understood, and very little is known about the enzymes that catalyze acetylation. Thus, the recent discovery of several types of genes that are required for acetylation opens up the possibility of evaluating whether this aspect of biomass composition can be altered [29,30].

The development of improved bioenergy crops poses significant challenges. Many of the perennial energy grasses are self-incompatible, making it difficult to produce true breeding lines. It is conceivable that this might be overcome by exploiting advances in understanding the molecular mechanisms of incompatibility, which might enable suppression. Conventional controlled crossing and screening of switchgrass has allowed identification of self-compatible and dihaploid stocks, which can enable production of inbred lines [86-87]. Where segregating populations are available, it should be possible to implement marker-assisted breeding to accelerate development of useful traits. Complete genome sequences are available for a number of important bioenergy crops, such as poplar [88], sorghum [89] and the model grass brachypodium [90], with sequencing of eucalyptus underway [91]. Genetic maps and partial sequences have also been generated for miscanthus [92] and sugarcane [93]. Since biomass accumulation and biomass digestibility are complex traits associated with many different pathways and genes, quantitative trait loci mapping has been used to identify contributing genetic factors in willow [94], poplar [95], sorghum [96], sugarcane [97], rice [53] and miscanthus [98].

Sustainability

It would be shortsighted to replace unsustainable petroleum production with unsustainable agricultural production of fuels. There is concern that current practices for production of major field crops are not sustainable because of factors such as declining aquifers, soil erosion, and energy intensive and environmentally damaging inputs of fertilizers and agrichemicals. Part of the interest in development of perennial energy crops arises from the evidence that such crops greatly diminish erosion, minimize inputs of minerals and lead to accumulation of soil carbon [36,37]. Thus, there appears to be an opportunity to develop biomass cropping systems that have positive impacts on agroecosystems. It is conceivable that because some prospective energy crops increase soil carbon, it may be possible to grow them in rotation with annual food crops that typically deplete soil carbon [37]. It is also possible that energy crops could be used to provide some ecosystem services that support conventional agriculture, such as providing refuge for insects that are targeted by Bt toxins or related technologies.

Because water is a major limitation of plant productivity, a key goal of developing bioenergy crops will be to maximize water use efficiency and drought tolerance for regions that do not receive excess rainfall. The aforementioned use of Agave species is particularly promising because such species can be up to ten-fold more water efficient than some C3 species and can be extremely drought tolerant. More generally, the use of perennial C4 grasses that have deep roots from the beginning of the growing season may allow significant production without irrigation in regions where annual crops require irrigation. There may also be opportunities to select for water-use efficiency traits [99].

The ability of some perennial species to recycle mineral nutrients on an annual basis by programmed senescence and mobilization of mineral nutrients from vegetative tissues to rhizomes for subsequent reuse is an extremely promising strategy for minimizing mineral inputs. By harvesting biomass after the senescence process, relatively low amounts of mineral nutrients are removed from the land. It follows that understanding the biological processes involved in mobilization and storage of nutrients is an important priority for future research. There have also been suggestions that some grasses are able to fix significant amounts of nitrogen without the involvement of nodules [37,100]. However, very little is known about the mechanism. If such mechanisms can be substantiated, they would seem to have implications for production of cereals that extend far beyond biofuels.

Concluding remarks

The topic of cellulosic biofuels is a complex subject with dimensions that prominently include economics, ecology, environmental sciences, agronomy, plant biology, microbiology, biochemistry, chemistry, genetics, chemical engineering, mechanical engineering, law and policy. In this brief commentary, we have highlighted some of the issues that are of most relevance to basic research on plants. Such advances have a crucial part to play in the expansion of biofuel production needed to help reduce the effect of fossil fuels on climate change.

Abbreviations

Cg1, corngrass1; EISA, Energy Independence and Security Act; FAO, Food and Agriculture Organization; RFS2, Renewable Fuel Standard.

Competing interests

This work is funded in part by a grant from BP.

Acknowledgements

This work was supported by the Energy Biosciences Institute.

References

1. Lewis NS, Nocera DG: **Powering the planet: chemical challenges in solar energy utilization.** *Proc Natl Acad Sci USA* 2006, **103:**15729-35.
2. Banerjee S, Fee R, Gopsteain A, Holland M, Hopkins A, Hummel H, Miner L: **Report on the first quadrennial technology review.** U.S. Department of Energy; 2011.
3. International Energy Agency (IEA): **Energy technology perspectives: Scenarios and strategies to 2050.** *OECD/IEA, Paris* 2010:706.
4. Heaton EA, Flavell RB, Mascia PN, Thomas SR, Dohleman FG, Long SP: **Herbaceous energy crop development: recent progress and future prospects.** *Curr Opin Biotechnol* 2008, **19:**202-209.
5. Karp A, Shield I: **Bioenergy from plants and the sustainable yield challenge.** *New Phytol* 2008, **179:**15-32.

6. Ragauskas AJ, Williams CK, Davison BH, Britovsek G, Cairney J, Eckert CA, Frederick WJ, Hallett JP, Leak DJ, Liotta CL, Mielenz JR, Murphy R, Templer R, Tschaplinski T: **The path forward for biofuels and biomaterials.** *Science* 2006, **311:**484.

7. Sticklen MB: **Plant genetic engineering for biofuel production: towards affordable cellulosic ethanol.** *Nat Rev Genet* 2008, **9:**433-43.

8. Carroll A, Somerville C: **Cellulosic biofuels.** *Annu Rev Plant Biol* 2009, **60:**165-82.

9. Perlack RD, Stokes BJ: **U.S. Billion-ton update: Biomass supply for bioenergy and bioproducts industry.** US Dept. Energy. Oak Ridge, TN: Oak Ridge National Laboratory; 2011:227.

10. Somerville C, Youngs H, Taylor C, Davis SC, Long SP: **Feedstocks for lignocellulosic biofuels.** *Science* 2010, **329:**790-2.

11. Campbell JE, Lobell DB, Genova RC, Field CB: **The global potential of bioenergy on abandoned agricultural lands.** *Environ Sci Technol* 2008, **42:**5791-4.

F1000 Factor 6
Evaluated by Chris Somerville 16 Apr 2012

12. Cai X, Zhang X, Wang D: **Land availability for biofuel production.** *Environ Sci Technol* 2011, **45:**334-9.

13. Amorim HV, Lopes ML, de Castro Oliveira JV, Buckeridge MS, Goldman GH: **Scientific challenges of bioethanol production in Brazil.** *Appl Microbiol Biotechnol* 2011, **91:**1267-75.

14. Goldemberg J: **The Brazilian biofuels industry.** *Biotech Biofuels* 2008, **1:**6.

F1000 Factor 6
Evaluated by Chris Somerville 16 Apr 2012

15. Brazilian Ministry of Agriculture - EMBRAPA: **Zoneamento Agroecológico de Cana-de-Açúcar.** *Presidential Decree No 6961* 2009.

16. Buckeridge MS, De Souza A, Arundale R, Anderson-Teixeira KJ, DeLucia EH: **Ethanol from sugarcane in Brazil: a "midway" strategy for increasing ethanol production while maximizing environmental benefits.** *GCB Bioenergy* 2012, **4:**119-26.

F1000 Factor 6
Evaluated by Chris Somerville 16 Apr 2012

17. Wallander S, Claassen R, Nickerson C: **The ethanol decade: An expansion of US corn production 2000-09.** Economic Information Bulletin 79: USDA Economic Research Service; 2011.

18. Mani S, Sokhansanj S, Tagore S, Turhollow AF: **Techno-economic analysis of using corn stover to supply heat and power to a corn ethanol plant - Part 2: Cost of heat and power generation systems.** *Biomass Bioenergy* 2010, **34:**356-64.

F1000 Factor 6
Evaluated by Chris Somerville 16 Apr 2012

19. Sokhansanj S, Mani S, Tagore S, Turhollow AF: **Techno-economic analysis of using corn stover to supply heat and power to a corn ethanol plant - Part 1: Cost of feedstock supply logistics.** *Biomass Bioenergy* 2010, **34:**75-81.

F1000 Factor 6
Evaluated by Chris Somerville 16 Apr 2012

20. Houghton J, Weatherwax S, Ferrell J: **Breaking the Barriers to Cellulosic Ethanol.** vol. DOE/SC-0095: U.S. Department of Energy; 2006.

21. Tao L, Aden A, Elander RT, Pallapolu VR, Lee YY, Garlock RJ, Balan V, Dale BE, Kim Y, Mosier NS, Ladisch MR, Falls M, Holtzapple MT, Sierra R, Shi J, Ebrik MA, Redmond T, Yang B, Wyman CE, Hames B, Thomas S, Warner RE: **Process and technoeconomic analysis of leading pretreatment technologies for lignocellulosic ethanol production using switchgrass.** *Bioresour Technol* 2011, **102:**11105-14.

22. Esteghlalian A, Hashimoto AG, Fenske JJ, Penner MH: **Modeling and optimization of the dilute-sulfuric-acid pretreatment of corn stover, poplar and switchhgrass.** *Bioresour Technol* 1997, **59:**129-36.

23. Himmel ME, Ding SY, Johnson DK, Adney WS, Nimlos MR, Brady JW, Foust TD: **Biomass recalcitrance: engineering plants and enzymes for biofuels production.** *Science* 2007, **315:**804-7.

F1000 Factor 6
Evaluated by Chris Somerville 16 Apr 2012

24. Hess M, Sczyrba A, Egan R, Kim TW, Chokhawala H, Schroth G, Luo S, Clark DS, Chen F, Zhang T, Mackie RI, Pennacchio LA, Tringe SG, Visel A, Woyke T, Wang Z, Rubin EM: **Metagenomic discovery of biomass-degrading genes and genomes from cow rumen.** *Science* 2011, **331:**463-7.

F1000 Factor 10
Evaluated by Chris Somerville 16 Apr 2012

25. Hahn-Hagerdal B, Karhumaa K, Jeppsson M, Gorwa-Grauslund MF: **Metabolic engineering for pentose utilization in Saccharomyces cerevisiae.** *Adv Biochem Eng Biotechnol* 2007, **108:**147-77.

26. Ha SJ, Galazka JM, Kim SR, Choi JH, Yang X, Seo JH, Glass NL, Cate JH, Jin YS: **Engineered Saccharomyces cerevisiae capable of simultaneous cellobiose and xylose fermentation.** *Proc Natl Acad Sci U S A* 2011, **108:**504-9.

F1000 Factor 6
Evaluated by Chris Somerville 16 Apr 2012

27. Petersson A, Almeida JR, Modig T, Karhumaa K, Hahn-Hagerdal B, Gorwa-Grauslund MF, Liden G: **A 5-hydroxymethyl furfural reducing enzyme encoded by the Saccharomyces cerevisiae ADH6 gene conveys HMF tolerance.** *Yeast* 2006: **23:**455-64.

F1000 Factor 6
Evaluated by Chris Somerville 16 Apr 2012

28. Qian X, Nimlos MR: **Mechanism of xylos and xylo-oligomer degradation during acid pretreatment.** In *Biomass recalcitrance.* Edited by Himmel ME. Oxford: Blackwell; 2008, 331-51.

29. Gille S, de Souza A, Xiong G, Benz M, Cheng K, Schultink A, Reca IB, Pauly M: **O-Acetylation of Arabidopsis hemicellulose xyloglucan requires AXY4 or AXY4L, proteins with a TBL and DUF231 domain.** *Plant Cell* 2011.

30. Manabe Y, Nafisi M, Verhertbruggen Y, Orfila C, Gille S, Rautengarten C, Cherk C, Marcus SE, Somerville S, Pauly M, Knox JP, Sakuragi Y, Scheller HV: **Loss-of-function mutation of REDUCED WALL ACETYLATION2 in Arabidopsis leads to reduced cell wall acetylation and increased resistance to Botrytis cinerea.** *Plant Physiol* 2011, **155:**1068-78.

31. Ranatunga TD, Jervis J, Helm RF, McMillan JD, Hatzis C: **Identification of inhibitory components toxic toward Zymomonas mobilis CP4(pZB5) xylose fermentation.** *Appl Biochem Biotechnol* 1997, **67:**185-98.

F1000 Factor 6
Evaluated by Chris Somerville 16 Apr 2012

32. Ranatunga TD, Jervis J, Helm RF, McMillan JD, Hatzis C: **Toxicity of hardwood extractives toward Saccharomyces cerevisiae glucose fermentation.** *Biotechnol Lett* 1997, **19:**1125-7.

F1000 Factor 6
Evaluated by Chris Somerville 16 Apr 2012

33. Lal R: **World crop residues production and implications of its use as a biofuel.** *Environ Int* 2005, **31:**575-84.

F1000 Factor 6
Evaluated by Chris Somerville 16 Apr 2012

34. Sokhansanj S, Turhollow A, Cushman J, Cundiff J: **Engineering aspects of collecting corn stover for bioenergy.** *Biomass Bioenergy* 2002, **23:**347-55.

35. Dohleman FG, Heaton EA, Leakey ADB, Long SP: **Does greater leaf-level photosynthesis explain the larger solar energy**

conversion efficiency of Miscanthus relative to switchgrass? *Plant Cell Environ* 2009, **32:**1525-37.

F1000 Factor 6
Evaluated by Chris Somerville 16 Apr 2012

36. Christian DG, Riche AB, Yates NE: **Growth, yield and mineral content of Miscanthus x giganteus grown as a biofuel for 14 successive harvests.** *Ind Crops Prod* 2008, **28:**320-7.

F1000 Factor 8
Evaluated by Chris Somerville 16 Apr 2012

37. Davis SC, Parton WJ, Dohleman FG, Smith CM, Del Grosso S, Kent AD, DeLucia EH: **Comparative biogeochemical cycles of bioenergy crops reveal nitrogen-fixation and low greenhouse gas emissions in a *Miscanthus x giganteus* agro-ecosystem.** *Ecosystems* 2010, **13:**144-56.
38. Heaton EA, Dohleman FG, Long SP: **Meeting US biofuel goals with less land: the potential of Miscanthus.** *Global Change Biol* 2008, **14:**2000-14.
39. Gonzalez-Hernandez JL, Sarath G, Stein JM, Owens V, Gedye K, Boe A: **A multiple species approach to biomass production from native herbaceous perennial feedstocks.** *In Vitro Cell Devel Biol-Plant* 2009, **45:**267-81.
40. Malmsheimer RW, Bowyer JL, Fried JS, Gee E, Izlar RL, Miner RA, Munn IA, Oneil E, Stewart WC: **Managing forests because carbon matters: Integrating energy, products, and land management policy.** *J Forestry* 2011, **109:**S7-48.
41. Goodale CL, Apps MJ, Birdsey RA, Field CB, Heath LS, Houghton RA, Jenkins JC, Kohlmaier GH, Kurz W, Liu S, Nabuurs GJ, Nilsson S, Shvidenko AZ: **Forest carbon sinks in the northern hemisphere.** *Ecol App* 2002, **12:**891-9.

F1000 Factor 6
Evaluated by Chris Somerville 17 Apr 2012

42. Campbell JE, Lobell DB, Genova RC, Field CB: **The global potential of bioenergy on abandoned agriculture lands.** *Environ Sci Technol* 2008, **42:**5791-4.
43. Tuskan GA: **Short-rotation woody crop supply systems in the United States: What do we know and what do we need to know?** *Biomass Bioenergy* 1998, **14:**307.

F1000 Factor 6
Evaluated by Chris Somerville 16 Apr 2012

44. Dornburg V, van Vuuren DP, van de Ven G, Langeveld H, Meeusen M, Banse M, van Oorschot M, Ros J, van den Born GJ, Aiking H, Londo M, Mozaffarian H, Verweij P, Lysen E, Faaij A: **Bioenergy revisited: Key factors in global potentials for bioenergy.** *Energy Environl Sci* 2010, **3:**258-67.
45. van Vuuren DP, van Vliet J, Stehfest E: **Future bio-energy potential under various natural constraints.** *Energy Policy* 2009, **37:**4220-330.
46. Pacca S, Moreira JR: **A biorefinery for mobility?** *Environ Sci Technol* 2011, **45:**9498-505.
47. Perlack RD, Wright LL, Turhollow AF, Graham RL, Stokes BJ, Erbach DC: **Biomass as feedstock for a biomass and bioproducts industry: the technical feasibility of a 1 billion ton annual feedstock supply.** Oak Ridge, TN: US Dept. Energy, Oak Ridge National Laboratory, Report ORNL/TM-2005/66; 2005:78.
48. Borland AM, Griffiths H, Hartwell J, Smith AC: **Exploiting the potential of plants with crassulacean acid metabolism for bioenergy production on marginal lands.** *J Exp Bot* 2009, **60:**2879-96.

F1000 Factor 6
Evaluated by Chris Somerville 16 Apr 2012

49. Nobel P: *Environmental Biology of Agaves and Cacti*. Cambridge UK: Cambridge University Press; 1988.
50. Subudhi PK, Baisakh N: **Spartina alterniflora Loisel., a halophyte grass model to dissect salt stress tolerance.** *In Vitro Cell Devel Biol-Plant* 2011, **47:**441-57.
51. Wicke B, Smeets E, Dornburg V, Vashev B, Gaiser T, Turkenburg W, Faaij A: **The global technical and economic potential of bioenergy from salt-affected soils.** *Energy Environ Sci* 2011, **4:**2669-81.

F1000 Factor 6
Evaluated by Chris Somerville 16 Apr 2012

52. Jakob K, Zhou FS, Paterson A: **Genetic improvement of C4 grasses as cellulosic biofuel feedstocks.** *In Vitro Cell Devel Biol-Plant* 2009, **45:**291-305.

F1000 Factor 8
Evaluated by Chris Somerville 16 Apr 2012

53. Jahn CE, Mckay JK, Mauleon R, Stephens J, Mcnally KL, Bush DR, Leung H, Leach JE: **Genetic Variation in biomass traits among 20 diverse rice varieties.** *Plant Physiol* 2011, **155:**157-68.
54. Demura T, Ye Z-H: **Regulation of plant biomass production.** *Curr Opin Plant Biol* 2010, **13:**299-304.
55. Ainsworth EA, Bush DR: **Carbohydrate export from the leaf: a highly regulated process and target to enhance photosynthesis and productivity.** *Plant Physiol* 2011, **155:**64-9.
56. Jin P, Guo T, Becraft P: **The maize CR4 receptor-like kinase mediates a growth factor-like differentiation response.** *Genesis* 2000, **27:**104-16.
57. Jin S, Kanagaraj A, Verma D, Lange T, Daniell H: **Release of hormones from conjugates: chloroplast expression of b-glucosidase results in elevated phytohormone levels associated with significant increase in biomass and protection from aphids or whiteflies conferred by sucrose esters.** *Plant Physiol* 2011, **155:**222-35.
58. Oh M-H, Sun J, Oh DH, Zielinski RE, Clouse SD, Huber SC: **Enhancing Arabidopsis leaf growth by engineering the BRASSINOSTEROID INSENSITIVE1 receptor kinase.** *Plant-Physiol* 2011, **157:**120-31.
59. Biemelt S: **Impact of altered gibberellin metabolism on biomass accumulation, lignin biosynthesis, and photosynthesis in transgenic tobacco plants.** *Plant Physiol* 2004, **135:**254-65.
60. Morinaka Y: **Morphological alteration caused by brassinosteroid insensitivity increases the biomass and grain production of rice.** *Plant Physiol* 2006, **141:**924-31.
61. Blachandran S, Hull RJ, Martins RA, Vaadia Y, Lucas WJ: **Influence of environmental stress on biomass partioning in transgenic tobacco plants expressing the movement protein of tobacco mosaic virus.** *Plant Physiol* 1997, **114:**475-81.
62. Vercruyssen L, Gonzalez N, Werner T, Schmulling T, Inze D: **Combining enhanced root and shoot growth reveals cross talk between pathways that control plant organ size in Arabidopsis.** *Plant Physiol* 2011, **155:**1339-52.
63. Salehi H, Ransom CB, Oraby HF, Seddighi Z, Sticklen MB: **Delay in flowering and increase in biomass of transgenic tobacco expressing the Arabidopsis floral respressor gene FLOWERING LOCUS C.** *J Plant Physiol* 2005, **162:**711-7.

F1000 Factor 6
Evaluated by Chris Somerville 16 Apr 2012

64. Maloof JN: **Recent advances in regulation of flowering.** *F1000 Biol Rep* 2010, **2**.

F1000 Factor 6
Evaluated by Chris Somerville 16 Apr 2012

65. Schnyder H, de Visser R: **Fluxes of reserve-derived and currently assimilated carbon and nitrogen in perennial ryegrass recovering from defoliation. The regrowing tiller and its component functionally distinct zones.** *Plant Physiol* 1999, **119:**1423-35.
66. Diaz C, Lemaitre T, Christ A, Azzopardi M, Kato Y, Sato F, Morot-Gaudry J-F, Le Dily F, Masclaux-Daubresse C: **Nitrogen recycling and remobilization are differentially controlled by leaf senescence and development stage in Arabidopsis under low nitrogen nutrition.** *Plant Physiol* 2008, **147:**1437-49.

67. An G-H, Miyakawa S, Kawahara A, Osaki M, Ezawa T: **Community structure of arbuscular mycorrhizal fungi associated with pioneer grass species Miscanthus sinensis in acid sulfate soils: Habitat segregation along pH gradients.** *Soil Sci Plant Nut* 2008, **54:**517-28.

68. Naidu SL: **Cold tolerance of C4 photosynthesis in Miscanthus x giganteus: Adaptation in amounts and sequence of C4 photosynthetic enzymes.** *Plant Physiol* 2003, **132:**1688-97.

F1000 Factor 6
Evaluated by Chris Somerville 16 Apr 2012

69. Prasifka JR, Bradshaw JD, Lee ST, Gray ME: **Relative feeding and development of armyworm on switchgrass and corn, and its potential effects on switchgrass grown for biomass.** *J Econ Ent* 2011, **104:**1561-7.

70. Tilman D, Reich PB, Knops JMH: **Biodiversity and ecosystem stability in a decade-long grassland experiment.** *Nature* 2006, **441:**629.

F1000 Factor 8
Evaluated by Andrew Hector 19 Jun 2006

71. Schmer MR, Vogel KP, Mitchell RB, Perrin RK: **Net energy of cellulosic ethanol from switchgrass.** *Proc Natl Acad Sci USA* 2008, **105:**464-9.

72. To JP, Zhu J, Benfey PN, Elich T: **Optimizing root system architecture in biofuel crops for sustainable energy production and soil carbon sequestration.** *F1000 Biol Rep* 2010:1-5.

73. Chen F, Dixon RA: **Lignin modification improves fermentable sugar yields for biofuel production.** *Nat Biotechnol* 2007, **25:**759-61.

74. Hu W-J, Harding SA, Lung J, Popko JL, Ralph J, Stokke DD, Tsai C-J, Chiang VL: **Repression of lignin biosynthesis promotes cellulose accumulation and growth in transgenic trees.** *Nature Biotechnol* 1999, **17:**808-12.

75. Kirst M, Myburg AA, De Leon JPG, Kirst M, Scott J, Sederoff R: **Coordinated genetic regulation of growth and lignin revealed by quantitative trait locus analysis of cdna microarray data in an interspecific backcross of eucalyptus.** *Plant Physiol* 2004, **135:**2368-78.

76. Ambavaram M, Krishnan A, Trijatmiko K, A. P: **Coordinated activation of cellulose and repression of lignin biosynthesis pathways in rice.** *Plant Physiol* 2011, **155:**916-31.

77. Novaes E, Kirst M, Chiang V, Winter-Sederoff H, Sederoff R: **Lignin and biomass: a negative correlation for wood formation and lignin content in trees.** *Plant Physiol* 2010, **154:**555-61.

78. Gallego-Giraldo L, Escamilla-Trevino L, Jackson LA, Dixon RA: **Salicylic acid mediates the reduced growth of lignin down-regulated plants.** *Proc Natl Acad Sci USA* 2011.

79. Hematy K, Sado PE, Van Tuinen A, Rochange S, Desnos T, Balzergue S, Pelletier S, Renou JP, Hofte H: **A receptor-like kinase mediates the response of Arabidopsis cells to the inhibition of cellulose synthesis.** *Curr Biol* 2007, **17:**922-31.

F1000 Factor 16
Evaluated by Gwyneth Ingram 07 Jun 2007, Simon McQueen-Mason 07 Jun 2007, Kay Schneitz 11 Jun 2007, Jan Traas 03 Jul 2007

80. Fu C, Mielenz JR, Xiao X, Ge Y, Hamilton CY, Rodriguez M, Jr., Chen F, Foston M, Ragauskas A, Bouton J, Dixon RA, Wang ZY: **Genetic manipulation of lignin reduces recalcitrance and improves ethanol production from switchgrass.** *Proc Natl Acad Sci USA* 2011, **108:**3803-8.

F1000 Factor 6
Evaluated by Chris Somerville 16 Apr 2012

81. Studer MH, DeMartini JD, Davis MF, Sykes RW, Davison B, Keller M, Tuskan GA, Wyman CE: **Lignin content in natural Populus variants affects sugar release.** *Proc Natl Acad Sci USA* 2011, **108:**6300-5.

F1000 Factor 6
Evaluated by Chris Somerville 16 Apr 2012

82. Grabber JH, Schatz PF, Kim H, Lu F, Ralph J: **Identifying new lignin bioengineering targets: 1. Monolignol-substitute impacts on lignin formation and cell wall fermentability.** *BMC Plant Biol* 2010, **10:**114.

83. Simmons BA, Loque D, Ralph J: **Advances in modifying lignin for enhanced biofuel production.** *Curr Opin Plant Biol* 2010, **13:**313-20.

F1000 Factor 6
Evaluated by Chris Somerville 16 Apr 2012

84. Garlock RJ, Balan V, Dale BE, Ramesh Pallapolu V, Lee YY, Kim Y, Mosier NS, Ladisch MR, Holtzapple MT, Falls M, Sierra-Ramirez R, Shi J, Ebrik MA, Redmond T, Yang B, Wyman CE, Donohoe BS, Vinzant TB, Elander RT, Hames B, Thomas S, Warner RE: **Comparative material balances around pretreatment technologies for the conversion of switchgrass to soluble sugars.** *Bioresour Technol* 2011, **102:**11063-71.

85. Chuck GS, Tobias C, Sun L, Kraemer F, Li C, Dibble D, Arora R, Bragg JN, Vogel JP, Singh S, Simmons BA, Pauly M, Hake S: **Overexpression of the maize Corngrass1 microRNA prevents flowering, improves digestibility, and increases starch content of switchgrass.** *Proc Natl Acad Sci U S A* 2011, **108:**17550-5.

86. Liu L, Wu Y: **Identification of a selfing compatible genotype and mode of inheritance in switchgrass.** *Bioenerg Res* 2011:1-7.

87. Young HA, Hernlem BJ, Anderton AL, Lanzatella CL, Tobias CM: **Dihaploid stocks of switchgrass isolated by a screening approach.** *Bioenerg Res* 2010, **3:**305-13.

88. Tuskan GA, Difazio S, Jansson S, Bohlmann J, Grigoriev I, Hellsten U, Putnam N, Ralph S, Rombauts S, Salamov A, Schein J, Sterck L, Aerts A, Bhalerao RR, Bhalerao RP, Blaudez D, Boerjan W, Brun A, Brunner A, Busov V, Campbell M, Carlson J, Chalot M, Chapman J, Chen GL, Cooper D, Coutinho PM, Couturier J, Covert S, Cronk Q, *et al*: **The Genome of black cottonwood, Populus trichocarpa (Torr. & Gray).** *Science* 2006, **313:**1596-604.

89. Paterson AH, Bowers JE, Bruggmann R, Dubchak I, Grimwood J, Gundlach H, Haberer G, Hellsten U, Mitros T, Poliakov A, Schmutz J, Spannagl M, Tang H, Wang X, Wicker T, Bharti AK, Chapman J, Feltus FA, Gowik U, Grigoriev IV, Lyons E, Maher CA, Martis M, Narechania A, Otillar RP, Penning BW, Salamov AA, Wang Y, Zhang L, Carpita NC, *et al*: **The Sorghum bicolor genome and the diversification of grasses.** *Nature* 2009, **457:**551-6.

90. The International Brachypodium Initiative: **Genome sequencing and analysis of the model grass Brachypodium distachyon.** *Nature* 2010, **463:**763-8.

F1000 Factor 6
Evaluated by Deborah Charlesworth 01 Mar 2010

91. Neale DB, Kremer A: **Forest tree genomics: growing resources and applications.** *Nature Rev Genet* 2011, **12:**111-22.

92. Glowacka K: **A review of the genetic study of the energy crop Miscanthus.** *Biomass Bioenergy* 2011, **7:**2445-54.

93. Garcia AAF, Kido EA, Meza AN, Souza HMB, Pinto LR, Pastina MM, Leite CS, Silva JAGD, Ulian EC, Figueira A, Souza AP: **Development of an integrated genetic map of a sugarcane (Saccharum spp.) commercial cross, based on a maximum-likelihood approach for estimation of linkage and linkage phases.** *Theor Appl Genet* 2006, **112:**298-314.

94. Brereton NJB, Pitre FE, Hanley SJ, Ray MJ, Karp A, Murphy RJ: **QTL mapping of enzymatic saccharification in short rotation coppice willow and its independence from biomass yield.** *Bioenerg Res* 2010, **3:**251-61.

F1000 Factor 6
Evaluated by Chris Somerville 17 Apr 2012

95. Rae AM, Pinel MPC, Bastien C, Sabatti M, Street NR, Tucker J, Dixon C, Marron N, Dillen SY, Taylor G: **QTL for yield in**

bioenergy Populus: identifying G×E interactions from growth at three contrasting sites. *Tree Genetics Genomes* 2007, **4:**97-112.

96. Shiringani AL, Friedt W: **QTL for fibre-related traits in grain × sweet sorghum as a tool for the enhancement of sorghum as a biomass crop.** *Theor Appl Genet* 2011, **123:**999-1011.

97. Pinto LR, Garcia AAF, Pastina MM, Teixeira LHM, Bressiani JA, Ulian EC, Bidoia MAP, Souza AP: **Analysis of genomic and functional RFLP derived markers associated with sucrose content, fiber and yield QTLs in a sugarcane (Saccharum spp.) commercial cross.** *Euphytica* 2010, **172:**313-27.

    F1000 Factor 6
    Evaluated by Chris Somerville 16 Apr 2012

98. Atienza SG, Satovic Z, Petersen KK, Martin A: **Identification of QTLs associated with yield and its components in *Miscanthus sinensis* Anderss.** *Euphytica* 2003, **132:**353-61.

99. Richards RA, Rebetzke GJ, Condon AG, van Herwaarden AF: **Breeding opportunities for increasing the efficiency of water use and crop yield in temperate cereals.** *Crop Sci* 2002, **42:**111-21.

100. Taulé C, Mareque C, Barlocco C, Hackembruch F, Reis VM, Sicardi M, Battistoni F: **The contribution of nitrogen fixation to sugarcane (*Saccharum officinarum* L.), and the identification and characterization of part of the associated diazotrophic bacterial community.** *Plant Soil* 2011.