Development of Energy Plants and their Potential to Withstand Various Extreme Environments

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Abstract: Biomass utilization is increasingly considered as a practical way for sustainable energy supply and long-term environment care around the world. In concerns with food security, starch or sugar-based bioethanol and edible-oil-derived biodiesel are severely restricted for large scale production. Alternatively, conversion of lignocellulosic residues from food crops could be considered, but due to its recalcitrance, the current biomass process is unacceptably expensive. In this context, genetic breeding of energy crops appears as a promising solution. To fulfil the global world need as both food and biofuel sources, energy crops are expected to be produced with higher yields and especially in marginal lands. This review focus on recent progress and patents dealing with energy plants and the challenges associated with bioenergy development. We also discuss the potential use of molecular approaches including genome sequencing, molecular markers, and genetic transformation for improving specific traits or generating new cultivars of energy plants.

Keywords: Abiotic stress, Biomass production, Biotechnology, Bioenergy, Cereals, Energy plants.

1. INTRODUCTION

Rapidly increasing energy demand has become a serious challenge both in developed and developing countries. Exploitation of renewable energy and sustainable energy is one of the effective solutions to this problem. Development of renewable energy can not only contribute to the energy supply, but also to achieve economic and environmental benefits [1]. Biomass energy is the most abundant and versatile type of renewable energy in the world [2]. In recent years, many countries have developed policies and objectives for bioenergy and this includes the production of heat, electricity, and fuel [3, 4]. Since 1975, Brazil has achieved greater energy security based on its focused commitment to developing competitive sugarcane industry and making ethanol a key part of its energy mix [3]. In fact, Brazil has replaced more than half of its gasoline needs with sugarcane ethanol- making gasoline the alternative fuel. Today, Brazil is the second biggest producer of ethanol in the world (20 billion litres) after the United States (24 billion litres). Close to 80 % of this production is for the domestic market- the fuel used in 45 % of Brazilian vehicles is ethanol. Many observers point to Brazil's experience as a case study for other nation's seeking to expand use of renewable fuels. The United States has used maize starch for bioethanol at 16.5 million Mg/year [5-8]. In China, starch bioethanol is mainly produced from the decayed and aged maize, rice and wheat grains at 1.33 million Mg/year [9]. As a contrast, lignocellulose ethanol production, because of its recalcitrance, is still under development [10,11]. In fact, a great effort has been made to increase the lignocellulose conversion rate, but the difficulty remains with two crucial factors: biomass pretreatment and enzymatic degradation. It is determined by cellulose crystallinity and lignin linking-styles of the plant cell walls [12,13]. In spite of extreme pretreatment conditions that can be a solution, such as strong acid/base, or extreme temperature/pressure, it leads to a negative economic profit of biofuel production together with a secondary environmental pollution [14]. Therefore, discovery of energy crops would provide a solution to a bottleneck situation. Without doubt, characterization of germplasm resources should be considered as an essential task to find out valuable genetic materials for energy crop breeding and to select high value energy plants.

Bioenergy crops can be classified as starch-producing crops, sugar-producing crops, lignocellulosic biomass crops, and oilseed crops for biodiesel production. Starch-producing crops include sweet potato [*Ipomoea batatas* L.] and cassava [*Manihot esculenta* Crantz.]; sugar-producing crops include sugarcane [*Saccharum officinarum* L.] and sweet sorghum [*Sorghum bicolor* [L.] Moench.]; lignocellulosic biomass crops include switchgrass [*Panicum virgatum* L.], miscanthus [e.g. *Miscanthus giganteus* Greef and Deu.] and poplar [*Populus spp*.]; and oilseed crops for biodiesel include soybean [*Glycine max* [L.] Merr.] and sunflower [*Helianthus annuus* L.]. Among the candidate energy crops, switchgrass, miscanthus, poplar, and sugarcane have been studied most extensively worldwide [3,15]. Energy plant resources differ from one geographic region to another according to their production capacity and their resistance to salt, drought, and/or low, high temperature stress. Plant species that have high production and are resistant to abiotic stress can be potentially developed as candidate energy plants. In this review, we report various strategies used to search alternatives to produce novel energetic forms, based on the valorisation of plant biomass. We reviewed recent progresses and patents

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of these potential bioenergy plants on their bioenergy traits related to abiotic stress.

2. BIOENERGY PLANTS

Bioenergy refers to a renewable energy derived from biological sources that can be used for heat, electricity, fuel and chemical products [3,16]. Principally, starch- and sugar-derived ethanol or plant oil-derived biodiesel is regarded as the first generation biofuel that has already made a relatively small but significant contribution to global energy supplies [5,17,18]. The second generation biofuel derived from lignocellulosic residues is predicted to be used in the near future [7,19,20].

To reach the bioenergy goal, biomass quality and quantity become crucial factors. Recently, the related topics have been extensively discussed on the biomass resources, ecological distribution, developmental history, and biofuel development policies of energy plants [9,21-25], but the underlying strategies have not been well described. Energy crops are potentially the largest source supply. Biomass yield of major energy crops are summarized in Table **1**. However, it is difficult to narrow down the potential estimate for this category since it mainly depends on two parameters that are very uncertain: [1] land availability, which depends on food sector development [food demand growth and agriculture productivity], demand for other agricultural and forestry commodities and factors constraining access to land, such as nature protection; and [2] the biomass yield levels that can be achieved on the available land. 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.

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 by-products. 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 [3]. Sorghum (*Sorghum bicolour*) 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 [3]. Two features had made 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 was particularly important for highly populated developing countries such as China and India. Second, sorghum is drought and heat tolerant, which would enable the usage of marginal land that was not suitable for the cultivation of many other crops [27]. Besides sorghum and maize, the residuals of other crops such as wheat and rice are also expected to be useful for lignocellulosic ethanol production.

Table 1. Biomass Yield of Energy Crops

Crop	Crop Yield (Fresh tonne/ha/yr)	Net Energy Yield in Fuel (Gj/ha/yr)
Corn	9.2	19-37
Wheat	5.1	15
Sorghum	1	19
Sugar-cane	73.1	84-152
Sugar-beet	58.5	111
Soy beans	2.7	12-13
Palm oil	19.2	140
Rape seed	2.9	28
Jatropha seeds	4-7	40
Woody crops, e.g. Poplar, Willow, Eucalyptus	10-15	90-110
Prairie grasses	3-6	18-28
Perennial herbaceous crops, e.g. Miscanthus, Switchgrass	10-30	140-230

Indicative biomass yields and possible subsequent transportation fuel production per hectare per year. Starch and sugar crops require conversion via fermentation to ethanol and oil crops to biodiesel via esterification (commercial technology at present). The woody and grass crops require either hydrolysis technology followed by ethanol or gasification to syngas to produce synthetic fuel [26].

Sugar-Producing Plants

Sugar can be used for direct fermentation of ethanol. Sugarcane (*Saccharum officinarum*) and sugar beet (*Beta vulgar*) 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 was a successful country that has reduced its gasoline usage by producing bioenergy. The Brazilian national ethanol program, which was based solely on sugarcane, produces 4.2 billion gallons of ethanol a year [28], although the resultant ecological and environmental effects are still debatable [29,30]. 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.

Bioenergy Feedstocks

Perennial bioenergy feedstocks are important sources of lignocellulosic biomass production. Switchgrass (*Panicum virgatu)* has been proposed as the major perennial feedstock in the USA because it was widely adapted, had high biomass production, high C-4 photosynthetic efficiency, and efficient use of water and nitrogen. Switchgrass yield was 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. 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 [31,32]. Another group of dedicated bioenergy feedstocks is woody plants, including hybrid poplar, willow and pines. Hybrid poplar was 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 was reported to be between 7 to 20 Mg/ha/yr depending on the nutrition and environmental conditions [33-35].

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 [36]. 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.

2. BIOENERGY PLANTS IMPROVEMENT FOR ABIOTIC STRESS TOLERANCE

Drought, heat, cold and salinity are among the major abiotic stresses that adversely affect plant growth and productivity, hence causing up to more than 50% crop yield loss worldwide. Water availability and water use efficiency are among the important abiotic factors that have a decisive influence on plant growth and development. Shortage of water resources and increased desertification are foreseen as consequences of the global warming [37]. Over 35% of the world's land surface is considered to be arid or semiarid, experiencing precipitation that is inadequate for most agricultural uses [38]. Regions that experience adequate precipitation can still be water limiting environments. Precipitation is rarely uniform. Agricultural regions affected by drought can experience yield loss up to 50% or more. These conditions will impose an increased evapo-transpiration to plants; therefore improving tolerance of agricultural system against abiotic stresses (drought, heat and salinity) represents a key trait to assure yield stability in the future. Developing crops that are more tolerant to water deficits while, maintaining productivity will become a critical requirement for enhancing agriculture in the future. Understanding how plant cells tolerate water loss is a vital prerequisite for developing strategies that can impact agricultural and horticultural crop productivity and survival under these conditions of decreasing water availability.

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 favourable 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 [39]. The upstream pathways for salt and drought stresses have been well-characterized in *Arabidopsis thaliana* [40-42], 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 [43,44]. 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 [45].

Drought tolerance is a complex trait, controlled by multiple genes. Gene switches such as transcription factors, which can have significant and specific effects on plant physiology, are desirable modulation targets. Stress-responsive NAC transcription factors (SNACs) may control expression of numerous downstream genes important for adaptation to drought stresses and may ultimately enhance drought tolerance through one or more mechanisms such as stomatal aperture reduction, delayed senescence, and increased sink and source strength. It has been shown that ABA- and drought-responsive NACs can enhance drought tolerance in *Arabidopsis* [46] and rice [47]. Therefore, SNAC genes from maize may also be used individually or in combination with other genes to enhance drought tolerance. The patent US20100287662 entitled "Maize stress-responsive nac transcription factors and promoter and methods of use" by Niu and Bates [48] had described the isolation and characterization of a promoter associated with a maize transcription factor ZmSNAC which can serve as a regulatory element for expression of isolated nucleotide sequences of interest, thereby impacting various traits in plants modulating root development, floral development, leaf and/or shoot development, senescence, seed size and/or weight, and tolerance of plants to abiotic stress.

Desirable loci can be introduced into commercially available plant varieties using marker-assisted selection (MAS) or marker-assisted breeding (MAB). MAS and MAB involves the use of one or more of the molecular markers for the identification and selection of those progeny plants that contain one or more loci coding for the desired traits. A patent US20110191892 entitled "Genetic markers associated with drought tolerance in maize" by Kishore *et al.* [49] had provided useful information's about molecular markers identified in maize and genetic improvement of tolerance to drought.

The Patent US20100138958 entitled "Plant responses" by Mullinbaux and Essex [50] was related to methods and uses for improving a plant's tolerance to abiotic or biotic stress. The method comprises introducing and overexpressing a polynucleotide sequence comprising or consisting of a plant Hsf into said plant. In particular, the invention had provided methods and uses for improving traits in plants which are important in the field of agriculture selected from the group comprising improved productivity, preferably growth or yield, water use efficiency, water productivity, drought tolerance or pathogen resistance.

In other patent dealing with transcription factor US20100192252 entitled "Transcription Factor Gene Induced by Water Deficit Conditions and Abscisic Acid from *Helianthus Annuus*, Promoter and Transgenic Plants" by Raquel *et al.* [51] detailed the isolation of a gene encoding for a transcription factor induced by water deficit or abscisic acid in *Helianthus annuus*. This gene has a homeo-domain associated with a leucine zipper. Transgenic plants overexpressing this transcription factor are tolerant and resistant to water and high salinity stresses.

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 showed large phenotypic variation for water and cold-stress tolerance even within cultivars [52-54]; 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.

3. MOLECULAR AND BIOTECHNOLOGICAL TOOLS TO IMPROVE ENERGY PLANTS

While conventional breeding methods continued to play an important role in developing new crops and cultivars, modern biotechnology tools are rapid and precise, and allow specific redesigning of crops for target characteristics. Recently, various biotechnologies have been used to improve the performance of energy plants. Biotechnology remains the key for reducing the cost and time for bioenergy conversion. Important genes related to production and stress tolerance may be discovered. For instance, over-expressing (or suppressing) some key genes to regulate energy conversion, enrichment, and distribution in the energy plants is promising.

Conventional Breeding Methods

Conventional breeding methods rely heavily on germplasm collection and conservation, and exploitation of germplasm resources. Methods such as mass selection, selection of pure lines, and conventional crossing are used to improve traits of commercial value. Recently, a collection of energy plants' germplasms has been established in China. The collection contains more than 100 known species [55]. Using inter-specific conventional cross to develop new high biomass producing varieties as well as highly level of tolerance to adverse conditions (drought, salinity, etc.), a number of F1 hybrids between *Saccharum officinarum* and *S. spontaneum* were obtained [56]. Some F1 hybrids were shown to produce very high dry matter up to 90 t/ha. These results showed promising potential for high biomass sugarcane varieties [56].

Molecular Markers

Molecular markers such as simple sequence repeats (SSR), random amplified polymorphic DNAs (RAPD), amplified fragment length polymorphisms (AFLP), single nucleotide polymorphisms (SNP), etc. have dramatically increased our understanding of the germplasm pool for energy crop species, and have increased our ability to characterize genetic diversity. For example, SSR markers were used to analyze the genetic diversity of 89 original cassava accessions and yielded 93 diverse loci. The data showed that genetic diversity in the total set of populations was small, and the average genetic diversity within populations was even smaller [57]. SSR markers were also used to determine genetic diversity among 32 sweet sorghum cultivars. A study using inter-simple sequence repeats (ISSR) showed that there is a high level of genetic diversity among barbados nut populations, and the genetic differentiation is lower between populations than within populations [58,59]. This basic information about genetic relationships among germplasm sources may guide the choice of parents for production of hybrids or improved populations [60].

Gene Identification

Key genes related to growth, development, metabolism, and stress resistance have been identified in several energy plants. For example, Wu *et al.* [61] isolated a full-length cDNA of an acyl-acyl carrier protein thioesterase [JcFATB1] from barbados nut. Specific overexpression of the JcFATB1 cDNA in *Arabidopsis thaliana* seed resulted in increased levels of saturated fatty acids and reduced levels of unsaturated fatty acids. The results suggest that JcFATB1 could potentially modify the seed oil of barbados nut to increase its levels of palmitate. A novel full-length cDNA encoding the water channel protein aquaporin was isolated from the seedlings of barbados nut. The results showed that the JcPIP2 protein is ubiquitously located in all tested tissues of the plant, and probably it played a role in drought resistance in barbados nut [62]. A key enzyme gene, SBEI, which played a role in the synthesis of branched starch, was also cloned. Manipulation of this gene was expected to inhibit synthesis of branching starch in cassava. This could be used to breed cultivars of cassava with a starch structure suited to processing into alcohol [63]. In addition, a novel transcription factor gene (JcERF) [64], a new plasma membrane intrinsic protein gene (JcPIP) [65], and a stearoyl-ACP desaturase gene (SAD) [66], have been cloned from barbados nut. In the future, these genes could be utilized in molecular breeding of these energy plants.

Genome Sequencing

Ongoing genomic research has made significant advances in characterization of genomes of plant crops including soybean [67], poplar [68], and sorghum [69], etc. This genome sequence information can be used to study and improve important agricultural traits in energy crops. Genome sequencing has become easier with the development of new-generation genome sequencing technologies. The GS FLX system (454 Life Sciences, Roche) can generate several hundred thousand DNA reads with an average length of 200-300 bases [70]. Completely genome sequences of some energy species were achieved like Soybean (46.430 genes [71]), Maize (32.000 genes [72]), Rapeseed (41.174 genes [73]) and Sorghum (27.640 genes [74]). With the development of transcriptomics and proteomics, dozens of genes and proteins are screened based on profiles of mRNA or protein expression. This makes it possible to identify a group of candidate genes that are putatively involved in expression of phenotypic and physiological traits [75]. The functions of genes can then be confirmed through genetic transformation [over-expression and/or knock-out studies] followed by trait analysis and search of allelic mutations [75].

''Omics'' Research

The accumulation of '-Omics' data had provided unprecedented conditions to study fundamental and practical issues in plant biology in a multidisciplinary manner. Genetic information on poplar and the nearly completed sequencing of the potato genome will progress research on energy trees and starch-producing crops respectively. Extensive genomic resources for corn and rice could be used for improving production in the grass family [76]. The sorghum genome is now being used as a template for resequencing native varieties of sweet sorghum. Comparative analysis of sweet sorghum and field sorghum genomes is expected to identify genes or regulatory elements responsible for increased sugar production [75]. Because sugarcane diverged from sorghum more recently (about 8-9 million years ago) than it diverged from maize and rice [about 12 and 45 million years ago respectively], the sorghum genome can be used as a template for assembling the autopolyploid genome of sugarcane [75]. To understand oil mobilization in germinating seeds, Yang *et al.* [77] performed proteomic analysis of endosperm in germinating barbados nut seeds. They found that 50 protein spots significantly changed in abundance during germination, indicating that several pathways were involved in oil mobilization.

Tissue Culture and Genetic Transformation of Energy Plants

Genetic engineering of the selected energy crops from the natural germplasm resource and mutagenesis pool is another challenge for further increasing biomass yield and biofuel production at a large scale. Because plant genetic engineering had played a major role in lignin modification, availability or establishment of a well defined, highly efficient transformation system for feedstock crops was an important prerequisite for the successful manipulation of lignin pathway genes to modify the quality or quantity of biomass [78]. Since most of the cellulosic feedstock crops are perennial grasses, which are considered recalcitrant for transformation procedures, the choice of transformation method will also have a great impact. To date, genetic transformation of plants has been performed by two main methods: Agrobacterium-mediated transformation and particle bombardment. The Agrobacterium method was originally used for dicotyledonous (dicot) plants such as tobacco, alfalfa, and poplar, natural hosts for Agrobacterium. After extensive studies, the Agrobacterium method has been extended to various monocotyledonous [monocot] plants including some feedstock species such as maize and switchgrass [79,80]. The early reports on grass transformation were mainly based on particle bombardment [81-83]. Considering the advantages of Agrobacterium-mediated transformation (lower copy number, fewer rearrangements of the transgene), it was the method of choice for transforming biofuel crops [80]. Genetically modified (GM) energy crop species may be more acceptable to the public than GM food crops, but there are still concerns about the potential environmental impacts of such plants, including gene flow from non-native to native plant relatives. It was important to note that, especially for restoration of degraded soils, bioenergy crops must be optimised not maximised, as low input systems involve limited nutrients and chemical inputs.

Mutation Induction of Energy Plant

With the development of genetic transformation techniques, antisense, RNA interference [RNAi], and virus-induced gene silencing [VIGS] have been used to knock down or silence the target gene[s] [84-86]. Genetic screens for mutants that affect cell wall composition and architecture, either directly or indirectly provided unbiased ways to identify biomass-relevant quality traits, including those resulting from mutations in cell wall-related genes. The brown mid-rib mutants of sorghum are an excellent example of how a defect in lignin structure, which improves forage digestibility by ruminants, can also enhance yields of glucose in screens using commercial cellulases [87]. In addition to Sorghum, brown-midrib mutants have been induced in other monocots such as maize and pearl millet that also showed a red-brown color of midribs with modified lignin composition and improved digestibility [88-90]. The bm mutants were good candidates for studying the relationship between lignin reduction and biofuel production. As more mutants are selected out, an integrated analysis in combination with the above natural germplasm information, can develop potential cell wall models that are refereed as selection standards of energy crop breeding [91].

4. PLANT BIOTECHNOLOGY SOLUTIONS FOR BIOENERGY

With the great effort in the world, significant progresses have been made to further improve bioenergy plants to benefit the production of bioenergy. Numerous studies and surveys have focused on the search for new biological sources and in particular those resulting from the biomass plant conversion and were illustrated by the results, which point to very important patents. 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 [92].

Modification of Lignin Biosynthesis

Lignin might be the most critical molecule in modification processes required for lignocellulosic feedstocks. It has been established that reducing lignin biosynthesis can lead to lower recalcitrance and higher saccharification efficiency [93-96]. Recent studies have indicated two important aspects for lignin modification. First, both lignin content and composition are important. Although it is co-dependent on efficient processes to fractionate lignin, a more uniform lignin structure might facilitate more efficient cell-wall degradation for fuel production. Second, the pre-treatment 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 [97]. Therefore, switchgrass, Miscanthus or poplar feedstocks with modified lignin can improve the efficiency of biomass conversion into fermentable sugars [97]. Although breeding plant biomass feedstock for reduced lignin content or increased biomass production will solve this problem [98], it will take a long time to achieve the goal. In this circumstance, modern biotechnological approaches offer great alternative opportunities to conventional plant breeding techniques to reduce the cost of cellulosic ethanol production [78]. The genetic engineering approaches include up-regulation of cellulose and hemicellulose pathway enzymes or other enzymes involved in increasing plant biomass characteristics or production of recombinant cellulases or hemicellulases in plants [99-103]. These approaches will possibly compensate the reduced saccharification efficiency due to the presence of lignin or minimize the use of enzymes during saccharification [104]. A direct and effective approach is to down-regulate the enzymes involved in lignin biosynthesis to reduce lignin content or to modify its composition [86,105,106].

Lignocellulose biomass, a source of fermentable sugar, has not been used on an industrial scale. In contrast to starch-containing raw materials such as cereals [maize, wheat, etc], enzymatic decomposition of the polysaccharides in the hemicellulose biomass to sugar is difficult. The resistance of the lignocellulose biomass to enzymatic hydrolysis occurs particularly at the crystalline phase of the cellulose, at the accessible surface, at the lignin coating and finally at the hemicelluloses screening the cellulose. The Patent US20110111474 entitled "Method for producing ethanol by fermentation from lignocellulosic biomass" by Karstens [107] relates to a method of producing bioethanol by separating lignin from a crushed lignocelluloses biomass and obtaining cellulose and, if required, hemicelluloses and further processing of the cellulose or mixture of cellulose and hemicellulose to obtain sugars and subsequently obtain bioethanol.

The patent US20080235820 entitled "Lignin reduction and cellulose increase in crop biomass via genetic engineering" by Masomeh and Sticklen [108] relate to a transgenic maize plant and methods of using the transgenic maize plant having at least a portion of a coding region of one or more lignin biosynthesis pathway enzymes. The transgenic plants use RNA interference [RNAi] to reduce lignin content or modify lignin residue configurations of the plants and increase cellulose.

Various plants and plant-derived materials are used for biofuel manufacturing. There are two common strategies of producing liquid and gaseous fuels. One is to grow crops with high sugar [sugar cane, sugar beet, and sweet sorghum] or starch [corn/maize] contents, and then use yeast fermentation to produce ethyl alcohol [ethanol]. The second is to grow plants that contain high amounts of vegetable oil, such as oil palm, soybean, algae, jatropha, or pongamia pinnata. When these oils are heated, their viscosity is reduced, and they can be burned directly in a diesel engine, or they can be chemically processed to produce fuels such as biodiesel. Wood and its byproducts can also be converted into biofuels such as methanol or ethanol fuel. The Patent US20110030097 entitled "Compositions and methods for enhancing oil content in plants" by Mayor and Rehovot [109] relates to microRNAs and related nucleic acid sequences associated with enhanced oil content in plants, as well as to methods for generating transgenic plants with enhanced oil content. The invention provides a transgenic plant transformed with an isolated nucleic acid selected from the group consisting of a microRNA, a microRNA precursor, a microRNA target, a microRNA-resistant target, a fragment thereof, a nucleic acid sequence capable of hybridizing thereto under stringent conditions, a nucleic acid sequence having at least 80% identity thereto, and combinations thereof, wherein modulated expression of the isolated nucleic acid sequence results in altered oil content in said transgenic plant compared to a corresponding wild type plant.

The patent US20110179525 entitled "Compositions and methods for biofuel crops" by Messing *et al.* [110] based on the use of the natural variation of sweet and grain sorghum to uncover genes that are conserved in rice, sorghum, and sugarcane, but differently expressed in sweet versus grain sorghum [by using a microarray platform and the syntenous alignment of rice and sorghum genomic regions containing these genes], the enzymes involved in carbohydrate accumulation and those that reduce lignocellulose can be identified. Interestingly, C4 photosynthesis is enhanced as well. The present invention relates to compositions and methods to increase the sugar content and/or decrease the lignocellulose content in plants such as corn, rice, sorghum, Brachypodium, Miscanthus and switchgrass. The invention involves identifying genes responsible for sugar and lignocellulose production and genetically altering the plants to produce biofuels in non-food plants as well as the non-food portions of food crop plants to use as biofuel.

Increasing Biomass Production and Yield

The importance of altering plant growth and development to increase the biomass production for bioenergy can not 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 [111-115]. Biotechnology could make rapid improvements in bioenergy feedstocks using genomics-guided improvements. For example, GA pathway genes such as Dgai [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 [116]. 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 [117]; dwarfing would increase the cellulosic content needed as feed, or for saccharification and fermentation needed for ethanol production. The lack of industrially suitable microorganisms for converting biomass into fuel ethanol has traditionally been cited as a major technical roadblock to developing bioethanol industry. In the last two decades, numerous microorganisms have been engineered to selectively produce ethanol [118]. Lignocellulosic biomass contains complex carbohydrates that necessitate utilizing microorganisms capable of fermenting sugars not fermentable by brewers' yeast. The most significant of these is xylose. The greatest successes have been in the engineering of Gram-negative bacteria: Escherichia coli, Klebsiella oxytoca, and Zymomonas mobilis. E. coli and K. oxytoca are naturally able to use a wide spectrum of sugars, and work has concentrated on engineering these strains to selectively produce ethanol. Z. mobilis produces ethanol at high yields, but ferments only glucose and fructose. Work on this organism has concentrated on introducing pathways for the fermentation of arabinose and xylose [118].

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 [119]. 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 [120]. Overall, the production of biomass can be further increased with the engineering of plant hormone response genes or genes involved in developmental processes [19,111,114,119,121,122].

The need on a global scale for energy crops as renewable fuels and alternative sources of farm income is of great importance to current ecological and economic issues. The fast growing warm season perennial, switchgrass [*Panicum virgantum* L.], has been identified as an ideal candidate for biomass fuel production. Switchgrass use as a bioenergy feedstock, in addition to providing energy, might reduce net carbon gas emissions, improve soil and water quality, increase native wildlife habitat, and increase farm revenues. Optimizing plant biomass for increased production and enhancing plant adaptation to adverse environments play important roles in cost-effective use of bioenergy. Interrelated plant traits such as higher yield, and resilience to biotic and abiotic challenge will increase industrial crop value in terms of biofuels and biomaterials. Genetically engineered switchgrass with enhanced biomass production and plant tolerance to abiotic stresses can be directly used for commercialization, benefiting the environment and energy security. The patent US20110197316 entitled “Methods and compositions for transgenic plants with enhanced abiotic stress resistance and biomass production” by Hong *et al.* [123] provides methods and compositions for producing transgenic plants having enhanced tolerance to biotic and/or abiotic stress and/or enhanced biomass production resulting from the expression of exogenous nucleotide sequences encoding SUMO E3 ligase or an active fragment thereof.

The patent US20110212835 entitled “Compositions and methods for increasing biomass, iron concentration, and tolerance to pathogens in plants” by Bais *et al.* [124] relate to the use of plant growth promoting rhizobacteria to enhance various characteristics of plant growth, including increasing biomass, increasing drought tolerance, decreasing lignin content, increasing seed germination, increasing iron concentration, and increasing tolerance to pathogens. The present invention relate to the administration of *Bacillus subtilis* FB17 to plants. The biomass of a plant which has been administered *Bacillus subtilis* FB17 can be converted to a biofuel or can be used as a food crop or in other uses.

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 [125-129]. Previous oilseed research has focused mainly on changing fatty acid profiles, particularly for nutritional purposes [127,129]. Recent efforts have also led to an increase in lipid production via induced expression of key exogenous lipid biosynthesis genes [128]. Metabolic engineering can also help to increase the production of sugar and starch for ethanol production using current platforms [130]. For example, recent research has indicated that the overexpression of a bacterial sucrose isomerase in vacuoles could double the sucrose yield for sugarcane [130]. 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.

5. IMPACT OF ENERGY PLANT PRODUCTION

In addition to providing a possible strategy for addressing the twin challenges of energy security and climate change, the production and use of bioenergy can also result in other [positive and negative] environmental, health and socioeconomic effects.

Environmental Impacts

Most of the environmental effects are linked to biomass feedstock production, many of which can be mitigated through best practices and appropriate regulation. Technical solutions are available for mitigating most environmental impacts from bioenergy conversion facilities, and their use was largely a question of appropriate environmental regulations and their enforcement. The use of organic waste and agricultural/forestry residues, and of lignocellulosic crops that could be grown on a wider spectrum of land types, may mitigate land and water demand and reduce competition with food.

The environmental impact from fuel processing is usually lower [131]. Bioenergy strategies that mainly focus on biofuels for transport and lead to increased cultivation of conventional agricultural crops for the production of 1st generation biofuels amplify the risk of further expansion of agricultural land into forests and other land with high biodiversity values, potentially causing continued ecosystem conversion and biodiversity loss [132]. They may also intensify concerns about the capacity of the agricultural resource base [soils, freshwater] to sustainably support an increasing agricultural output, due to the well-documented degradation of soils and water bodies that typically accompanies intensive agricultural practices [133,134]. 1st generation biofuels faced both social and environmental challenges, largely because they use food crops which could lead to food price increases and possibly indirect land use change. While such risks can be mitigated by regulation and sustainability assurance and certification, technology development is also advancing for next generation processes that rely on non-food biomass [e.g. lignocellulosic feedstocks such as organic wastes, forestry residues, high yielding woody or grass energy crops and algae]. The use of these feedstocks for 2nd generation biofuel production would significantly decrease the potential pressure on land use; improve greenhouse gas emission reductions when compared to some 1st generation biofuels, and result in lower environmental and social risk.

Li *et al.* [135] assessed the potential for CO_2 emission reduction by developing non grain based ethanol crops. The results show that non-grain based bio-ethanol production can potentially reduce CO_2 emissions from the 2007 levels by 11 million tons and 49 million tons in 2015 and 2030, respectively [5.5 and 25 times of the reduction capacity in 2007]. Further, growing bioenergy crops on marginal lands may also affect methane emissions. Fu and Yu [136] indicate that methane emissions in China rose at an annual rate of 2% from 1990 to 2006 due to increases in rice cultivation, livestock populations, and field burning of crop residues. Overall, the studies by this group suggest that development of bioenergy crops may change China's greenhouse gas emissions due to land use changes and an increase in animal food supply requirements [e.g., grasses for both forage and biofuel]. In addition to improving environmental sustainability and security while reducing concerns for food, economic gains can also be made when bioenergy development is integrated with ecological restoration. For example, many non-food bioenergy plants are perennials with deep root systems. Ecological restoration using these plants can thus lead to an increase in land productivity for food [as compared to the tilled farming system] in terms of improvements in soil and water conservation. Bioenergy-driven restoration of degraded ecosystems can also increase terrestrial carbon sequestration due to large biomass production and root residues as well as slowing decomposition of soil organic materials under no-till conditions.

Economical and Social Impact

Bioenergy sustainability depends upon farmers making the correct decisions about land use patterns and the adoption of energy plants. In general, many countries have a bioenergy market of billions of dollars per year. However, given a competitive economic environment, it is unclear whether biomass energy can be economically sustainable. Currently, more than 60% of the cost for bioenergy is in feedstock costs [137]. This provides an opportunity for farmers to increase their income. A potential risk of high feedstock cost is that it may cause an increase in land conversion from food to fuel production, eventually causing a food-energy conflict. To avoid this problem, a basic premise is that cultivating bioenergy crops must not infringe on grain supply and marketing. This requires not only breakthroughs in biotechnology for biomass productivity but also effective coordination between farmers and industry through bioenergy certification. Utilization of marginal lands for bioenergy can also increase the economic contribution of bioenergy because the costs for ecological restoration can be reduced. Biomass production on marginal/degraded land may not be the automatic outcome of increasing biomass demand. As bioenergy use increases and farmers adopt bioenergy crops, they will consider developments in both the food and bioenergy sectors when planning their operations. The economic realities at farm level may then still lead to bioenergy crops competing with food crops, since it is the good soils that also result in higher yields for the bioenergy crops. Biomass plantations may eventually be pushed to marginal/degraded land due to increasing land costs following increased competition for prime cropland, but this competition will probably also be reflected in increasing food commodity prices. The development of bioenergies will affect the market of food commodities, change the modalities of managing the resources [conflict for the utilization of the soil and water] and influence the social and economic development, even outside the agricultural sector. Co-firing of coal and biomass (e.g., 10-20% biomass) in power plants (referred to as biocoal) is another approach to benefiting farmers economically while producing clean energy. This is because the addition of biomass to coal can create an effective mechanism that allows farmers to share the profits of the coal energy industry. As for the societal impacts of bioenergy development, the understanding of bioenergy economics is important but it is not sufficient [138]. Psychological, cognitive, and cultural factors, which shape a farmer's values and beliefs, are just as important as economics. All these economic and social factors must be dealt with at the policy level. Policy around bioenergy needs to be designed so that it is consistent with meeting environmental and social objectives. Bioenergy needs to be regulated so that environmental and social issues are taken into consideration, environmental services provided by bioenergy systems are recognized and valued, and it contributes to rural development objectives.

6. CURRENT AND FUTURE DEVELOPMENTS

Bioenergy is already making a substantial contribution to meeting global energy demand. This contribution can be expanded very significantly in the future, providing greenhouse gas savings and other environmental benefits, as well as contributing to energy security, improving trade balances, providing opportunities for social and economic development in rural communities, and improving the management of resources and wastes. Biotechnological techniques have made it possible to produce energy plants with desirable traits. As energy plants become increasingly important worldwide, research will be directed at enhancing production, quality, and resistance, while reducing costs and minimizing any adverse effects on the environment. Further research will be required to achieve these goals. Firstly, energy plant resources have to be evaluated systematically. Secondly, genomic studies need to be carried out so that molecular techniques, e.g. DNA markers, can be developed. In addition, modern genetic engineering technologies such as genetic transformation will be used to improve energy plants. The development of a sustainable bioenergy industry will necessitate a better understanding of the risks posed by this growing sector and the development of practices and policies that minimise any environmental and social risks and maximise the multi-functional benefits that biomass can provide. The public needs to be informed and confident that bioenergy is environmentally and socially beneficial and does not result in significant negative environmental and social trade-offs. However, the industry is confident such challenges can be met as similar challenges have been addressed in other sectors and appropriate technologies and practices are being developed and deployed. The debate around bioenergy plants has often proved emotional in recent years. There is a need for this debate to become more informed by sound scientific evidence. This also means that more consistent approaches to assessing the impacts and opportunities of bioenergy are required.

CONFLICT OF INTEREST

We confirm that we do not have conflict of interest with any research team working on bioenergy plants and environmental constraints.

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