Recent Progress in the Genetic Engineering of Biofuel Crops

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Kinya Akashi and Yoshihiko Nanasato

Abstract

The utilization of biomass energy is increasingly considered as a promising means for the sustainable supply of energy and for long-term conservation of the global environment. In order to achieve the effective production of biomass-based energy, a key challenge will be the breeding of biofuel crops that enable high and stable biomass production. In this context, genetic engineering to optimize metabolism, create value-added biomass production, and enable environmental adaptability for growth on marginal land will be instrumental for establishing the next generation of biofuel crops. This review focuses on recent progress in the development of dedicated biofuel crops by means of genetic engineering, particularly switchgrass for lignocellulosic feedstock and jatropha and camelina for biodiesel feedstock.

Keywords

Arid region • Biofuel crop • Biomass production • Genetic engineering • Transgenic plants

K. Akashi (✉)
School of Agricultural, Biological and Environmental Sciences, Faculty of Agriculture, Tottori University, 4-101 Koyama Minami, Tottori 680-8553, Japan
e-mail: akashi.kinya@muses.tottori-u.ac.jp

Y. Nanasato
Forestry and Forest Products Research Institute Forest Bio-Research Center, 3809-1 Ishi, Juo, Hitachi, Ibaraki 319-1301, Japan

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18.1 Introduction

Global energy demand has increased rapidly in the last decade, and securing a sustainable energy supply for the future constitutes an urgent challenge both in developed and developing countries. Owing to its abundance, accessibility, versatility, and renewability, plant biomass energy has been recognized as a potential solution for this issue (Yuan et al. 2008). Bioenergy from various biofuel crops is already making an important contribution to meet global energy demand, in the form of heat, electricity, and transport fuels (Dudley 2014). Based on the type of principal chemical compound used for energy productions, biofuel crops can be classified into four groups:

- Sugar-producing crops, e.g., sugarcane (*Saccharum officinarum*) and sweet sorghum (*Sorghum bicolor*)
- Starch-producing crops, e.g., maize (*Zea mays*) and cassava (*Manihot esculenta*)
- Lignocellulosic biomass crops, e.g., switchgrass (*Panicum virgatum*), miscanthus (*Miscanthus giganteus*), and poplar (*Populus* spp.)
- Oilseed crops for biodiesel, e.g., soybean (*Glycine max*), canola (*Brassica napus*), camelina (*Camelina sativa*), and jatropha (*Jatropha curcas*)

Currently, a large proportion of biofuel feedstock is derived from sugar- or starch-producing edible crops, which could potentially cause competition with food production over resources such as lands and water. In contrast, bioethanol production from lignocellulose, which is a more abundant resource, remains to be fully developed. This is partly because of the recalcitrant nature of lignocellulose, which requires harsh physicochemical pretreatments and/or enzymatic degradation before ethanol fermentation and, therefore, a higher associated cost. Technical innovation to overcome these limitations has been one of the central issues in biofuel research. Other challenges in biofuel development include the generation of more productive biofuel crops and the expansion of cultivation areas to unused arid lands. The development of biofuel crops with higher yields and better stress resistance is expected to play a central role in this process of innovation. Together with fundamental research into germplasm exploration and conventional breeding, genetic engineering has been increasingly recognized as an alternative approach for the improvement of agronomical traits in biofuel crops (Fig. 18.1). In this chapter, we focus on technological innovations in the genetic engineering of biofuel crops that have been applied to improve various aspects of their agronomic traits (Table 18.1).

18.2 Development for Genetic Engineering in Biofuel Crops

To explore the potential of genetic engineering for biofuel crops, the establishment of efficient transformation protocols in a given plant species is of crucial importance. Plant transformation procedures are composed of several elaborate steps

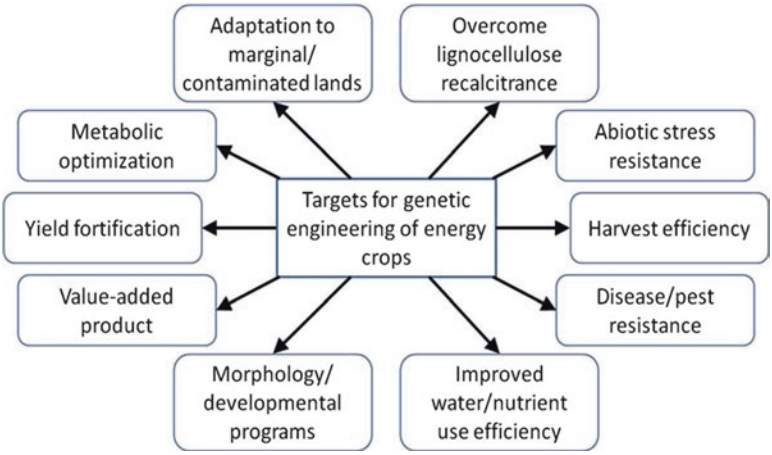

Fig. 18.1 Targets for genetic engineering of energy crops. Sustainable and profitable production of energy crops depends on the availability of varieties with suitable agronomic traits, and genetic engineering potentially contributes to the enhancement of existing genotypes to maximize the productivity of large-scale biofuel production

including design of foreign gene vector constructs, gene delivery into the selected plant tissues, regeneration of the transformed cell/tissues, and the selection of stable transgenic plants with the desired traits. Because the establishment of a transformation procedure requires extensive effort to optimize the experimental conditions for each step, it is not surprising that transformation technologies are relatively more advanced in traditional edible crops with longer research histories, such as maize (Rhodes et al. 1988; Que et al. 2014), soybean (Chee et al. 1989; Homrich et al. 2012), sorghum (Howe et al. 2006; Girijashankar and Swathisree 2009), and sugarcane (Manickavasagam et al. 2004; Mayavan et al. 2013).

Encouragingly, in most of the newly proposed biofuel crops, major progress has been made recently in the development and optimization of transformation protocols. Recently established examples include those for switchgrass (Somleva et al. 2002; King et al. 2014), miscanthus (Wang et al. 2011), camelina (Lu and Kang 2008), and jatropha (Li et al. 2008; Kajikawa et al. 2012). In most cases, tissue culture techniques and/or plant regeneration protocols are prerequisite for the establishment of genetic transformation protocols. Methods of choice for gene delivery into plant cells can vary depending on the plant species and associated culture conditions: In miscanthus, foreign gene delivery was performed using a particle bombardment-mediated transformation system (Wang et al. 2011). For jatropha (Li et al. 2008; Kajikawa et al. 2012) and camelina (Lu and Kang 2008), many research groups have successfully employed *Agrobacterium*-mediated transformation. In camelina, a vacuum-infiltration-assisted floral dip transformation protocol has been developed (Lu and King 2008), which allowed high-throughput generation of transgenic plants in this species. In switchgrass, both *Agrobacterium*-mediated (Somleva

Table 18.1 Examples of genetic engineering in representative energy crops

Crop	Target gene	Engineering type	Target traits	References
Switchgrass				
PHB biosynthetic genes (*phaABC*)		Overexpression	Bioplastic production	Somleva et al. (2008)
Cinnamyl alcohol dehydrogenase (*CAD*)		RNAi	Lignin modification	Saathoff et al. (2011)
Caffeic acid *O*-methyltransferase (*COMT*)		RNAi	Lignin modification	Fu et al. (2011)
4-Coumarate-CoA ligase (*4CL*)		RNAi	Lignin modification	Xu et al. (2011)
miR156		Overexpression	Plant development	Fu et al. (2012)
LONG VEGETATIVE PHASE 1 (*LOV1*)		Overexpression	Plant development	Xu et al. (2012)
Jatropha				
Delta 12-desaturase (*FAD2*)		RNAi	Lipid composition	Qu et al. (2012)
Vacuolar Na^+/H^+ antiporter (*NHX1*)		Overexpression	Salinity resistance	Jha et al. (2013)
Curcin		RNAi	Toxin reduction	Patade et al. (2014)
FLOWERING LOCUS T (*FT*)		Overexpression	Early flowering	Li et al. (2014)
SUGAR-DEPENDENT 1 (*SDP1*)		RNAi	Lipid yield	Kim et al. (2014)
Bt endotoxin Cry1Ab/1Ac		Overexpression	Insect resistance	Gu et al. (2014)
Camelina				
Purple acid phosphatase 2 (*PAP2*)		Overexpression	Lipid yield	Zhang et al. (2012)
P_{1B}-ATPase (*HMA*)		Overexpression	Metal tolerance	Park et al. (2014)
MYB96		Overexpression	Wax fortification	Lee et al. (2014)
Gγ-subunit of G-protein (*AGG3*)		Overexpression	Lipid yield	Choudhury et al. (2014)
Fatty acid elongase (*KCS3*)		Overexpression	HFA production	Snapp et al. (2014)

The table depicts transgenic plants reported in dedicated biofuel crops (switchgrass, jatropha, and camelina), type of genetic engineering, and their target agronomic traits. For more detailed descriptions, see text

et al. 2002) and particle bombardment-mediated transformation (King et al. 2014) approaches have been reported.

Although *Agrobacterium*-mediated stable genetic transformation is well established in model plants, it remains less efficient and more technically demanding for most biofuel crops. One of the drawbacks of *Agrobacterium*-mediated gene transfer is production of the plant stress hormone ethylene during infection, which impairs transformation efficiency by repressing vir gene expression (Nonaka et al. 2008). To

improve the transformation efficiency, a gene for 1-aminocyclopropane-1-carboxylate (ACC) deaminase was introduced into *Agrobacterium* cells for the purpose of ethylene decomposition. Consequently, gene transfer efficiency was improved in recalcitrant energy crops including canola (Hao et al. 2010) and a cellulosic biofuel crop *Erianthus ravennae* (Someya et al. 2013), the latter represents a wild relative of sugarcane and shows very high dry matter production.

In addition to the development of efficient genetic transformation methods, a series of molecular tools are required to establish transgenic plant varieties for practical use. A reliable gene promoter that directs transgene expression in a desired tissue with sufficient strength is a necessity. Conventional gene promoters such as cauliflower mosaic virus (CaMV) 35S and rice ubiquitin 2 (*ubi2*) promoters often fail to deliver sufficiently high transgene expression levels in transgenic biofuel crops. To overcome this limitation, polyubiquitin gene promoters were isolated and analyzed using a series of promoter-GUS fusion constructs in transgenic switchgrass, which led to the characterization of a new promoter element that directed strong transgene expression in this energy crop (Mann et al. 2011). Increasing the repertoire of these molecular tools will further increase the versatility of genetic engineering in biofuel crops.

For the commercial production of transgenic biofuel crops in the field, technical development and practical guidelines are needed to prevent the dispersal of transgenic pollen into the environment. Technological innovations to control pollen-mediated gene flow from transgenic plants have made significant progress in recent years (Sang et al. 2013), and these technological concepts are now being applied to various biofuel crops. The Bxb1-*att* recombination system is composed of a Bxb1 enzyme and its recognition sequences *attP* and *attB*, and these components execute unidirectional site-specific recombination without the need for cofactors (Yau et al. 2011). This system was introduced into switchgrass using pollen-specific promoters, and the specific removal of marker genes from switchgrass pollen and generation of transgene-excised progeny was demonstrated (Somleva et al. 2014). In other studies, a Cre-*lox*-mediated recombination system was introduced into transgenic jatropha for the purpose of marker gene excision (Qu et al. 2012), and marker-free transgenic jatropha expressing Bt-endotoxin Cry1Ab/1Ac protein for lepidopteran insect resistance was generated using this technique (Gu et al. 2014).

18.3 Metabolic Engineering

Biodiesel in the form of fatty acid methyl esters is produced by the transesterification of triacylglycerols (TAGs) with methanol in the presence of acid or alkali. TAGs are abundant in plant seeds, which serve as excellent sources of biodiesel feedstock (Durrett et al. 2008). The quality of biodiesel is highly dependent on the composition of fatty acids and other ingredients in seed storage organs. To optimize biodiesel production, not only increasing the oil yield in plant seeds but also

engineering the plant metabolism for the optimal composition of fatty acids and other ingredients are required.

In plants, the carbon source for seed TAG biosynthesis is derived from sucrose, a product of photosynthesis in the leaves. TAG biosynthesis starts in the seed plastids where fatty acid chains are elongated, and the intermediates are transported to the endoplasmic reticulum where acyl-CoAs are converted to diacylglycerols (DAGs) (Durrett et al. 2008). DAGs are then converted to TAGs by diacylglycerol acyltransferase (DGAT), a committing step for TAG biosynthesis from the membrane lipid biosynthetic pathway. Knowledge obtained from research in model plants such as *Arabidopsis* is now being applied to metabolic engineering in practical crop cultivars used for biofuels. For example, overexpression of a fungal DGAT2 enzyme resulted in a 1.5% increase in oil content in soybean seeds (Lardizabal et al. 2008). The molecular properties of DGATs were investigated in jatropha, which showed increased seed oil levels when expressed in transgenic *Arabidopsis* (Misra et al. 2013).

TAGs contain three fatty acid chains per molecule, each of which is esterified to a glycerol backbone. Thus, the supply of glycerol-3-phosphate influences TAG biosynthesis. Indeed, overexpression of the yeast glycerol-3-phosphate dehydrogenase (*ghpd1*) gene increased the lipid content in canola seeds by 40% (Vigeolas et al. 2007), suggesting that carbon flux into the glycerol backbone is also important for increasing oil accumulation in seeds.

An alternative approach for increasing TAG abundance in seeds is to suppress its degradation. *Sugar-dependent 1* (SDP1) is a specific lipase that regulates the first step of TAG catabolism (Eastmond 2006). Expression of a *SDP1* homolog was suppressed in oilseed rape (*Brassica napus*), which resulted in an increase in oil yield in these transgenic plants (Kelly et al. 2013). A similar strategy was undertaken for a jatropha homolog, *JcSDP1*, by RNA interference (RNAi) technology, which resulted in a 13–30% increase in the total seed lipid content, at the expense of a 7% decrease in protein content in transgenic jatropha seeds (Kim et al. 2014).

Optimization of the fatty acid composition with the aim of developing desirable physicochemical properties for biodiesel fuels is also an important research target (Durrett et al. 2008). A monounsaturated oleic acid (C18:1) is a preferred component as an acyl chain in TAGs, because of its high cetane value, low melting point, and resistance to oxidation. In soybean, modulation of the fatty acid composition was achieved by suppressing the expression of FATB, an acyl-ACP thioesterase, which led to the accumulation of oleic acid up to 85% compared to 18% in the wild type (Buhr et al. 2002). In jatropha, when the gene encoding delta 12-desaturase (*FAD2*), which catalyzes the conversion of oleic acid to linoleic acid (C18:2), was suppressed by RNAi, it achieved a significant increase in oleic acid up to 78% and a corresponding reduction in polyunsaturated fatty acids in the transgenic seeds (Qu et al. 2012).

In addition to usage as a fuel, there has been a growing interest in the use of biomass materials as other industrial feedstocks, including for plastics and chemicals. Polyhydroxyalkanoate (PHA) polymers occur in nature in some microbes as a storage reserve (Anderson and Dawes 1990), and PHA-based bioplastics have been

increasingly used in a various commercial products (Snell and Peoples 2009). Pathways for PHA production have been introduced into various crops by a transgenic approach (Suriyamongkol et al. 2007), including switchgrass (Somleva et al. 2008; McQualter et al. 2014).

18.4 Lignocellulose Engineering

Lignocellulose in plant cell walls is the most abundant biomaterial on earth. It is composed of three major polymers, the polysaccharides cellulose and hemicellulose and the phenolic polymer lignin. (Pauly and Keegstra 2008; Sticklen 2008; Zhao et al. 2012). Although technological innovation in the chemical and/or biological conversion of lignocellulose into liquid fuels has advanced considerably in recent years (Zhao et al. 2012), the processes still require harsh physicochemical pretreatments to extract monosaccharides from this polymer, and these are associated with higher capital and operating costs. To design biofuel crops with improved lignocellulosic characteristics for more efficient breakdown, research has focused on the fundamental aspects of biosynthesis and degradation of plant cell walls, and this new understanding is now being applied to the molecular breeding of biofuel crops.

Among lignocellulose components, lignin is recognized as one of the major factors responsible for cell wall recalcitrance for biofuel conversion (Weng et al. 2008). Lignin polymers are composed of several phenylalanine-derived lignin monomers (or monolignols), which are synthesized by the phenylpropanoid pathway in the cytoplasm. The monomers are transported across the plasma membrane to the cell wall, where they undergo oxidative coupling and crosslinking with polysaccharides components of the cell wall (Bonawitz and Chapple 2010). Initial attempts to lower the lignin level by transgenic approaches resulted in growth penalties in plants, suggesting the pivotal role of the phenylpropanoid pathway in plant development and productivity. Transgenic alfalfa, in which genes for the phenylpropanoid pathway downregulated, showed increased hydrolysis of cell wall polysaccharides, but this change was accompanied by dwarfism, low biomass yield, and severe growth defects (Chen and Dixon 2007). Interestingly, individual transgenic plants with different steps in the phenylpropanoid pathway blocked were phenotypically diverse and did not show a correlation with lignin accumulation levels (Bonawitz and Chapple 2013 for review). These observations indicated the pleiotropic roles of this metabolic pathway on plant development and biotic/abiotic stress resistance, which are often exerted in a plant species-specific manner. These studies highlighted the importance of the elaborate design required for lignin engineering and the need to achieve lignin modification without penalty in growth and productivity.

Genetic engineering of lignin modification has been attempted in practical biofuel crops, which gave rise to intriguing results. Caffeic acid *O*-methyltransferase (*COMT*) catalyzes *O*-methylation of the 5-hydroxyl groups of monolignol precursors, thereby functioning in the latter step of the lignin biosynthetic pathway. RNAi-mediated downregulation of was conducted in model plant species, leading to a decrease in the S/G lignin monomer ratio and total lignin content without

detrimental effects on plant growth (Guo et al. 2001; Jung et al. 2012). This strategy was introduced into switchgrass, and downregulation of *COMT* resulted in a 30–38% increase in ethanol yield per unit biomass compared with controls (Fu et al. 2011). Subsequently, the performance of *COMT*-downregulated transgenic switchgrass plants was analyzed in the field for two growing seasons, and higher sugar release and ethanol production with no apparent growth penalties were observed (Baxter et al. 2014). It was estimated that a 50% increase in liters of ethanol per hectare can be achieved using these transgenic plants, demonstrating the potential of lignin engineering to overcome the limitations of biofuel production with standard source crops.

18.5 Stress Resistance

Abiotic stresses such as water deficit and high salinity are recognized as one of the most influential factors for plant growth and crop productivity. Thus, improving the environmental resistance of biofuel crops represents a key challenge to expand their cultivation zones to drought-, heat-, and/or salinity-affected marginal land. To meet this challenge, a number of stress resistance genes have been tested, intransgenic biofuel crops. The vacuolar Na^+/H^+ antiporter (*NHX1*) functions in the sequestration of excess Na^+ into the vacuole, thereby contributing to Na^+ homeostasis and salinity stress resistance in plants (Apse et al. 1999; Blumwald 2000). A *Nhx1* gene from the extreme halophyte *Salicornia brachiate* was introduced into jatropha (Jha et al. 2013), and transgenic lines exhibited enhanced tolerance to 200 mM NaCl in the growth media, thereby demonstrating the effectiveness of this approach. In another study, transgenic jatropha plants with drought-resistance genes were generated, including phosphopantetheine adenylyltransferase (*PPAT*) for coenzyme A biosynthesis, the B subunit of the nuclear factor Y (*NF-YB*) transcription factor, and the *GSMT/DMT* genes for the biosynthesis of compatible solute glycine betaine (Tsuchimoto et al. 2012). Further physiological analysis of these transgenic plants will evaluate the effectiveness of the transgenes on drought stress resistance. The transcription factor MYB96 regulates foliar cuticle wax biosynthesis and is implicated in drought resistance (Seo et al. 2011). Overexpression of this gene was attempted in camelina, which resulted in fortification of foliar cuticle layer and improved drought resistance (Lee et al. 2014).

Soil contamination by heavy metals represents a serious environmental issue in many regions of the world and impairs plant growth and productivity. The P1B-ATPase (*HMA*) gene family is implicated in the homeostasis and detoxification of heavy metals in plants (Williams and Mills 2005). Genomic and transcription analyses have identified a heavy-metal-responsive *CsHMA3* gene from camelina, and *CsHMA3* overexpression in transgenic camelina showed improved heavy metal tolerance (Park et al. 2014).

18.6 Developmental Regulation

The morphological and developmental traits of plants, such as those for the aboveground canopy and root system architecture, vegetative/reproductive transition, and leaf/flower/fruit development, have strong impacts on the biomass yield of biofuel crops (Jakob et al. 2009). Knowledge obtained from model plants such as *Arabidopsis* is now being applied to practical energy crops, which has given rise to promising results. The *FLOWERING LOCUS T* (*FT*) gene is one of the central factors integrating flowering signals and plays a crucial role in the transition from vegetative phase to flowering (Wigge 2011). A homolog gene *JcFT* was isolated from jatropha, and transgenic jatropha overexpressing this gene exhibited a significant early flowering phenotype (Li et al. 2014). It is expected that such introduced traits will be helpful for improving the otherwise unreliable and poor flowering trait in this plant.

In contrast to the case of jatropha, in which its seeds are utilized for biodiesel production, a longer vegetative phase and late flowering would be a preferred trait in the case of switchgrass, because lignocellulose in the vegetative tissues is utilized for bioethanol production. *LONG VEGETATIVE PHASE 1* (*LOV1*) encodes a NAC-type transcription factor and contributes to cold resistance and a delayed flowering phenotype under long-day conditions in *Arabidopsis* (Yoo et al. 2007). Overexpression of the *Arabidopsis LOV1* gene led to pleiotropic effects in transgenic switchgrass, such as smaller leaf angle, altered lignin content and monolignol compositions in the cell wall, and delayed flowering (Xu et al. 2012), demonstrating the potential advantages of developmental engineering in biofuel crops.

In plants, microRNA-156 (miR156) is a member of a gene family of small, noncoding RNAs with a higher expression level in the juvenile phase of development (Wu et al. 2009). Expression of most members of the *SQUAMOSA PROMOTER-BINDING PROTEIN-LIKE* (*SPL*) transcription factor family, which are implicated in diverse developmental processes such as leaf development, shoot maturation, and flowering, is regulated by miR156 in a Dicer-like protein (DCL1)-dependent manner (Xing et al. 2010; Gou et al. 2011). In transgenic switchgrass, overexpression of endogenous miR156b resulted in diverse developmental changes such as altered apical dominance, later flowering, and increased biomass yield (Fu et al. 2012), suggesting the potential of morphological alteration for improving the biomass yield of energy crops.

18.7 Concluding Remarks

In recent decades, there have been significant developments in the technological aspects of genetic engineering of energy crops. Using these experimental systems, a number of transgenic energy crops have been generated with the aim of improving various aspects of their agronomic traits. These new plants have been subjected to evaluation by a range of criteria including technological effectiveness and stability, socioeconomic benefits, and environmental sustainability. Considering many of the

promising results presented in this chapter, it has been forecast that innovation in genetically engineered biofuel crops will accelerate further in the future. Moreover, in the next phase, pyramiding of multiple transgenes conferring combined traits in a single plant is expected. Furthermore, promotion of field studies using these genetically engineered biofuel crops, with the aim of commercial GMO-based biofuel production, is anticipated for the successful adaptation of plant biotechnology to the biofuel industry.

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References

Anderson AJ, Dawes EA (1990) Occurrence, metabolism, metabolic role, and industrial uses of bacterial polyhydroxyalkanoates. Microbiol Rev 54:450–472

Apse MP, Aharon GS, Snedden WA, Blumwald E (1999) Salt tolerance conferred by over expression of a vacuolar Na^+/H^+ antiport in Arabidopsis. Science 285:1256–1258

Baxter HL, Mazarei M, Labbe N, Kline LM, Cheng Q, Windham MT, Mann DGJ, Fu C, Ziebell A, Sykes RW, Rodriguez M Jr, Davis MF, Mielenz JR, Dixon RA, Wang Z-Y, Stewart CN Jr (2014) Two-year field analysis of reduced recalcitrance transgenic switchgrass. Plant Biotechnol J 12:914–924

Blumwald E (2000) Sodium transport and salt tolerance in plants. Curr Opin Cell Biol 12:431–434

Bonawitz ND, Chapple C (2010) The genetics of lignin biosynthesis: connecting genotype to phenotype. Annu Rev Genet 44:337–363

Bonawitz ND, Chapple C (2013) Can genetic engineering of lignin deposition be accomplished without an unacceptable yield penalty? Curr Opin Biotechnol 24:336–343

Buhr T, Sato S, Ebrahim F, Xing A, Zhou Y, Mathiesen M, Schweiger B, Kinney A, Staswick P (2002) Ribozyme termination of RNA transcripts down-regulate seed fatty acid genes in transgenic soybean. Plant J 30:155–163

Chee PP, Fober KA, Slightom JL (1989) Transformation of soybean (*Glycine max*) by infecting germinating seeds with *Agrobacterium tumefaciens*. Plant Physiol 91:1212–1218

Chen F, Dixon RA (2007) Lignin modification improves fermentable sugar yields for biofuel production. Nat Biotechnol 25:759–761

Choudhury R, Riesselman AJ, Pandey S (2014) Constitutive or seed-specific overexpression of Arabidopsis G-protein γ subunit 3 (AGG3) results in increased seed and oil production and improved stress tolerance in *Camelina sativa*. Plant Biotechnol J 12:49–59

Dudley B (2014) BP statistical review of world energy. http://www.bp.com/statisticalreview

Durrett TP, Benning C, Ohlrogge J (2008) Plant triacylglycerols as feedstocks for the production of biofuels. Plant J 54:593–607

Eastmond PJ (2006) SUGAR-DEPENDENT 1 encodes a patatin domain triacylglycerol lipase that initiates storage oil breakdown in germinating Arabidopsis seeds. Plant Cell 18:665–675

Fu C, Mielenz JR, Xiao X, Ge Y, Hamilton CY, Rodriguez M, Chen F, Foston M, Ragauskas A, Bouton J, Dixon RA, Wang ZY (2011) Genetic manipulation of lignin reduces recalcitrance and improves ethanol production from switchgrass. Proc Natl Acad Sci USA 108:3803–3808

Fu C, Sunkar R, Zhou C, Shen H, Zhang J-Y, Matts J, Wolf J, Mann DGJ, Stewart CN Jr, Tang Y, Wang Z-Y (2012) Overexpression of miR156 in switchgrass (*Panicum virgatum* L.) results in various morphological alterations and leads to improved biomass production. Plant Biotechnol J 10:443–452

Girijashankar V, Swathisree V (2009) Genetic transformation of *Sorghum bicolor*. Physiol Mol Biol Plants 15:287–302

Gou J-Y, Felippes FF, Liu C-J, Weigel D, Wang J-W (2011) Negative regulation of anthocyanin biosynthesis in Arabidopsis by a miR156-targeted SPL transcription factor. Plant Cell 23:1512–1522

Gu K, Mao H, Yin Z (2014) Production of marker-free transgenic *Jatropha curcas* expressing hybrid *Bacillus thuringiensis* δ-endotoxin Cry1Ab/1Ac for resistance to larvae of tortrix moth (*Archips micaceanus*). Biotechnol Biofuels 7:68

Guo D, Chen F, Inoue K, Blount JW, Dixon RA (2001) Downregulation of caffeic acid 3-O-methyltransferase and caffeoyl CoA 3-O-methyltransferase in transgenic alfalfa: impacts on lignin structure and implications for the biosynthesis of G and S lignin. Plant Cell 13:73–88

Hao Y, Charles TC, Glick BR (2010) ACC deaminase increases the *Agrobacterium tumefaciens*-mediated transformation frequency of commercial canola cultivars. FEMS Microbiol Lett 307:185–190

Homrich MS, Wiebke-Strohm B, Weber RLM, Bodanese-Zanettini MH (2012) Soybean genetic transformation: a valuable tool for the functional study of genes and the production of agronomically improved plants. Genet Mol Biol 35:998–1010

Howe A, Sato S, Dweikat I, Fromm M, Clemente T (2006) Rapid and reproducible *Agrobacterium*-mediated transformation of sorghum. Plant Cell Rep 25:784–791

Jakob K, Zhou F, Paterson A (2009) Genetic improvement of C4 grasses as cellulosic biofuel feedstocks. In Vitro Cell Dev Biol Plant 45:291–305

Jha B, Mishra A, Jha A, Joshi M (2013) Developing transgenic Jatropha using the SbNHX1 gene from an extreme halophyte for cultivation in saline wasteland. PLoS One 8:e71136

Jung JH, Fouad WM, Vermerris W, Gallo M, Altpeter F (2012) RNAi suppression of lignin biosynthesis in sugarcane reduces recalcitrance for biofuel production from lignocellulosic biomass. Plant Biotechnol J 10:1067–1076

Kajikawa M, Morikawa K, Inoue M, Widyastuti U, Suharsono S, Yokota A, Akashi K (2012) Establishment of bispyribac selection protocols for *Agrobacterium tumefaciens*- and *Agrobacterium rhizogenes*-mediated transformation of the oil seed plant *Jatropha curcas* L. Plant Biotechnol 29:145–153

Kelly AA, Shaw E, Powers SJ, Kurup S, Eastmond PJ (2013) Suppression of the SUGAR-DEPENDENT 1 triacylglycerol lipase family during seed development enhances oil yield in oilseed rape (*Brassica napus* L.) Plant Biotechnol J 11:355

Kim MJ, Yang SW, Mao HZ, Veena SP, Yin JL, Chua NH (2014) Gene silencing of sugar-dependent 1 (JcSDP1), encoding a patatin-domain triacylglycerol lipase, enhances seed oil accumulation in *Jatropha curcas*. Biotechnol Biofuels 7:36

King ZR, Bray AL, LaFayette PR, Parrott WA (2014) Biolistic transformation of elite genotype of switchgrass. Plant Cell Rep 33:313–322

Lardizabal K, Effertz R, Levering C, Mai J, Pedroso MC, Jury T, Aasen E, Gruys K, Bennett K (2008) Expression of *Umbelopsis ramanniana* DGAT2A in seed increases oil in soybean. Plant Physiol 148:89–96

Lee SB, Kim H, Kim RJ, Suh MC (2014) Overexpression of Arabidopsis MYB96 confers drought resistance in *Camelina sativa* via cuticular wax accumulation. Plant Cell Rep 33:1535–1546

Li M, Li H, Jiang H, Pan X, Wu G (2008) Establishment of an *Agrobacterium*-mediated cotyledon disc transformation method for *Jatropha curcas*. Plant Cell Tissue Organ Cult 92:173–181

Li C, Luo L, Fu Q, Niu L, Xu Z-F (2014) Isolation and functional characterization of JcFT, a FLOWERING LOCUS T (FT) homologous gene from the biofuel plant *Jatropha curcas*. BMC Plant Biol 14:125

Lu C, Kang J (2008) Generation of transgenic plants of a potential oilseed crop *Camelina sativa* by *Agrobacterium*-mediated transformation. Plant Cell Rep 27:273–278

Manickavasagam M, Ganapathi A, Anbazhagan VR, Sudhakar B, Selvaraj N, Vasudevan A, Kasthurirengan S (2004) Agrobacterium-mediated genetic transformation and development of herbicide-resistant sugarcane (*Saccharum* species hybrids) using axillary buds. Plant Cell Rep 23:134–143

Mann DGJ, King ZR, Liu W, Joyce BL, Percifield RJ, Hawkins JS, LaFayette PR, Artelt BJ, Burris JN, Mazarei M, Bennetzen JL, Parrott WA, Stewart CN Jr (2011) Switchgrass (*Panicum virgatum* L.) polyubiquitin gene (PvUbi1 and PvUbi2) promoters for use in plant transformation. BMC Biotechnol 11:74

Mayavan S, Subramanyam K, Arun M, Rajesh M, Kapil Dev G, Sivanandhan G, Jaganath B, Manickavasagam M, Selvaraj N, Ganapathi A (2013) *Agrobacterium tumefaciens*-mediated in planta seed transformation strategy in sugarcane. Plant Cell Rep 32:1557–1574

McQualter RB, Somleva MN, Gebbie LK, Li X, Petrasovits LA, Snell KD, Nielsen LK, Brumbley SM (2014) Factors affecting polyhydroxybutyrate accumulation in mesophyll cells of sugarcane and switchgrass. BMC Biotechnol 14:83

Misra A, Khan K, Niranjan A, Nath P, Sane VA (2013) Over-expression of JcDGAT1 from *Jatropha curcas* increases seed oil levels and alters oil quality in transgenic *Arabidopsis thaliana*. Phytochemistry 96:37–45

Nonaka S, Sugawara M, Minamisawa K, Yuhashi K, Ezura H (2008) 1-Aminocyclopropane-1-carboxylate deaminase enhances *Agrobacterium tumefaciens*-mediated gene transfer into plant cells. Appl Environ Microbiol 74:2526–2528

Park W, Feng Y, Ahn S-J (2014) Alteration of leaf shape, improved metal tolerance, and productivity of seed by overexpression of CsHMA3 in *Camelina sativa*. Biotechnol Biofuels 7:96

Patade VY, Khatri D, Kumar K, Grover A, Kumari M, Gupta SM, Kumar D, Nasim M (2014) RNAi mediated curcin precursor gene silencing in Jatropha (*Jatropha curcas* L.) Mol Biol Rep 41:4305–4312

Pauly M, Keegstra K (2008) Cell-wall carbohydrates and their modification as a resource for biofuels. Plant J 54:559–568

Qu J, Mao H-Z, Chen W, Gao S-Q, Bai Y-N, Sun Y-W, Geng Y-F, Ye J (2012) Development of marker-free transgenic Jatropha plants with increased levels of seed oleic acid. Biotechnol Biofuels 5:10

Que Q, Elumalai S, Li X, Zhong H, Nalapalli S, Schweiner M, Fei X, Nuccio M, Kelliher T, Gu W, Chen Z, Chilton MDM (2014) Maize transformation technology development for commercial event generation. Front Plant Sci 2014:379

Rhodes CA, Pierce DA, Mettler IJ, Mascarenhas D, Detmer JJ (1988) Genetically transformed maize plants from protoplasts. Science 240:204–207

Saathoff AJ, Sarath G, Chow EK, Dien BS, Tobias CM (2011) Downregulation of cinnamyl-alcohol dehydrogenase in switchgrass by RNA silencing results in enhanced glucose release after cellulose treatment. PLoS One 6:e16416

Sang Y, Millwood RJ, Neal Stewart C Jr (2013) Gene use restriction technologies for transgenic plant bioconfinement. Plant Biotechnol J 11:649–658

Seo PJ, Lee SB, Suh MC, Park MJ, Go YS, Park CM (2011) The MYB96 transcription factor regulates cuticular wax biosynthesis under drought conditions in Arabidopsis. Plant Cell 23:1138–1152

Snapp AR, Kang J, Qi X, Lu C (2014) A fatty acid condensing enzyme from *Physaria fendleri* increases hydroxy fatty acid accumulation in transgenic oilseeds of *Camelina sativa*. Planta 240:599–610

Snell KD, Peoples OP (2009) PHA bioplastic: a value-added coproduct for biomass biorefineries. Biofuel Bioprod Bior 3:456–467

Someya T, Nonaka S, Nakamura K, Ezura H (2013) Increased 1-aminocyclopropane-1-carboxylate deaminase activity enhances *Agrobacterium tumefaciens*-mediated gene delivery into plant cells. Microbiol Open 2:873–880

Somleva MN, Tomaszewski Z, Conger BV (2002) Agrobacterium-mediated genetic transformation of switchgrass. Crop Sci 42:2080–2087

Somleva MN, Snell KD, Beaulieu JJ, Peoples OP, Garrison BR, Patterson NA (2008) Production of polyhydroxybutyrate in switchgrass, a value-added co-product in an important lignocellulosic biomass crop. Plant Biotechnol J 6:663–678

Somleva MN, Xu CA, Ryan KP, Thilmony R, Peoples O, Snell KD, Thomson J (2014) Transgene autoexcision in switchgrass pollen mediated by the Bxb1 recombinase. BMC Biotechnol 14:79

Sticklen MB (2008) Plant genetic engineering for biofuel production: towards affordable cellulosic ethanol. Nat Rev Genet 9:433–443

Suriyamongkol P, Weselake R, Narine S, Moloney M, Shah S (2007) Biotechnological approaches for the production of polyhydroxyalkanoates in microorganisms and plants – a review. Biotechnol Adv 25:148–175

Tsuchimoto S, Cartagena J, Khemkladngoen N, Singkaravanit S, Kohinata T, Wada N, Sakai H, Morishita Y, Suzuki H, Shibata D, Fukui K (2012) Development of transgenic plants in Jatropha with drought tolerance. Plant Biotechnol 29:137–143

Vigeolas H, Waldeck P, Zank T, Geigenberger P (2007) Increasing seed oil content in oil-seed rape (*Brassica napus* L.) by overexpression of a yeast glycerol-3-phosphate dehydrogenase under the control of a seed-specific promoter. Plant Biotechnol J 5:431–441

Wang X, Yamada T, Kong F-J, Abe Y, Hoshino Y, Sato H, Takamizo T, Kanazawa A, Yamada T (2011) Establishment of an efficient *in vitro* culture and particle bombardment-mediated transformation system in *Miscanthus sinensis* Anderss., a potential bioenergy crop. GCB Bioenergy 3:322–332

Weng JK, Li X, Bonawitz ND, Chapple C (2008) Emerging strategies of lignin engineering and degradation for cellulosic biofuel production. Curr Opin Biotechnol 19:166–172

Wigge PA (2011) FT, a mobile developmental signal in plants. Curr Biol 21:R374–R378

Williams LE, Mills RF (2005) P(1B)-ATPases – an ancient family of transition metal pumps with diverse functions in plants. Trends Plant Sci 10:491–502

Wu G, Park MY, Conway SR, Wang J-W, Weigel D, Poethig RS (2009) The sequential action of miR156 and miR172 regulates developmental timing in Arabidopsis. Cell 138:750–759

Xing S, Salinas M, Hohmann S, Berndtgen R, Huijser P (2010) miR156-targeted and nontargeted SBP-Box transcription factors act in concert to secure male fertility in Arabidopsis. Plant Cell 22:3935–3950

Xu B, Escamilla-Treviño LL, Sathitsuksanoh N, Shen Z, Shen H, Zhang YH, Dixon RA, Zhao B (2011) Silencing of 4-coumarate:coenzyme A ligase in switchgrass leads to reduced lignin content and improved fermentable sugar yields for biofuel production. New Phytol 192:611–625

Xu B, Sathitsuksanoh N, Tang Y, Udvardi MK, Zhang J-Y, Shen Z, Balota M, Harich K, Zhang PYH, Zhao B (2012) Overexpression of AtLOV1 in switchgrass alters plant architecture, lignin content, and flowering time. PLoS One 7:e47399

Yau YY, Wang Y, Thomson JG, Ow DW (2011) Method for Bxb1-mediated site-specific integration *in planta*. Methods Mol Biol 701:147–166

Yoo SY, Kim Y, Kim SY, Lee JS, Ahn JH (2007) Control of flowering time and cold response by a NAC-domain protein in Arabidopsis. PLoS One 2:e642

Yuan JS, Tiller KH, Al-Ahmad H, Stewart NR, Stewart CN (2008) Plants to power: bioenergy to fuel the future. Trends Plant Sci 13:421–429

Zhang Y, Yu L, Yung KF, Leung DY, Sun F, Lim BL (2012) Over-expression of AtPAP2 in *Camelina sativa* leads to faster plant growth and higher seed yield. Biotechnol Biofuels 5:19

Zhao X, Zhang L, Liu D (2012) Biomass recalcitrance. Part I: the chemical compositions and physical structures affecting the enzymatic hydrolysis of lignocellulose. Biofuels Bioprod Biorefining 6:465–482