

Genetic improvement of C4 grasses as cellulosic biofuel feedstocks

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Abstract C4 grasses are among the most productive plants and most promising cellulosic biofuel feedstocks. Successful implementation of cellulosic biofuel feedstocks will depend on the improvement of critical crop characteristics and subsequent conversion technologies. The content and composition of lignin, cellulose, and hemicellulose, their biomass yields, and their biotic and abiotic stress tolerances are critical factors which can be enhanced by molecular breeding methods, including marker-assisted selection and transgenic approaches. To maximize biomass yield, no flowering or late flowering and no grain set would be ideal for cellulosic biofuel crops. Reducing fecundity also reduces the risk of undesired gene transfer and invasiveness, thus accelerating deregulation processes and permitting faster implementation of highly improved genotypes in cellulosic feedstock production.

Keywords Biomass yield · Lignin · Germplasm · *Miscanthus* · Switchgrass · *Sorghum* · SSRs · SNPs · QTL · Marker-assisted selection · Transcription factor · Transgenic

Why Do We Need to Develop Cellulosic Biofuels?

The hunt for carbon neutral energy sources has become one of the primary challenges of the twenty-first century. The

use of ethanol is a proven concept for replacing gasoline and reducing CO₂ emission from fossil oil. Currently, ethanol is produced from sugar- and starch-rich crops, which are grown by labor- and machine-intensive agriculture and require high-nitrogen fertilization. These practices in turn negatively impact the overall energy and CO₂ balance for this particular production chain. Cellulosic biofuels are a promising component in a future mix of alternative renewable energy solutions. There are challenges which exist in the use of cellulosic biofuel crops; but with continuously developing plant breeding, crop development, and farming as well as conversion technologies, cellulosic biofuel crops will emerge as strong contenders in the race for sustainable energy sources. Among the many choices for cellulosic biofuels are trees and short rotation coppice, agricultural waste material, and by-products from crops and biomass grasses. This review will focus on the genetic improvement of selected C4 grasses including *Sorghum bicolor*, *Miscanthus* species, hybrids between these species, and hybrids between *Miscanthus* × sugarcane (Miscane), and *Panicum virgatum* (switchgrass) for the development of dedicated cellulosic biofuel crops.

Characteristics of Sustainable Cellulosic Biofuel Crops

The transition from the Mesozoic to Neolithic period (around 10,000 BCE) is defined by the development of nascent agricultural practices and the selection and genetic manipulation of cereal grasses to meet the qualitative and quantitative requirements of food and feedstock. In this respect, the development of cellulosic biofuel feedstock will be similar. Tailoring crops for biofuel production is critical in improving the overall economy of ethanol production from cellulosic feedstock (Wyman 2007). Lignin content

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and composition of cell walls determine, among other factors, sugar release and thus the efficiency of the ethanol extraction process (Davison et al. 2006; Chen and Dixon 2007; Vermerris et al. 2007; Yoshida et al. 2008). Hence, biomass composition, in particular cell wall composition, plays an important role and is considered a major trait for improvement of cellulosic biofuels (Sticklen 2006; McCann and Carpita 2008). Lignin, cellulose, and hemicellulose are main components of cell walls and their biosynthesis is facilitated by complex pathways. While the lignin content and biosynthesis pathway have been extensively studied for digestibility purposes for animal feedstock like corn, other grasses, and alfalfa (Jung and Vogel 1986; Baucher et al. 1999; Barrière et al. 2004; Ralph et al. 2004; Reddy et al. 2005) and for improving pulping qualities in wood (Hu et al. 1999; Pilate et al. 2002; Baucher et al. 2003; Li et al. 2003), we are only beginning to unravel the details of cellulose and hemicellulose biosynthesis (Paredez et al. 2006; DeBolt et al. 2007; Desprez et al. 2007; Lindeboom et al. 2008; Paredez et al. 2008; Wang et al. 2008) and the regulation of these pathways (Mitsuda et al. 2005; Zhong et al. 2006; Mitsuda et al. 2007; Zhong et al. 2007a, b; Zhong and Ye 2007; Xu et al. 2008; Zhong et al. 2008). Reliable and accurate measurements for cell wall components are labor intensive and standards are not consistently established (Kelley et al. 2004; Labbé et al. 2008) which complicate the exact definition of the most favorable ratio of lignin, cellulose, and hemicellulose in the cell wall for ethanol production. Given the complexity, cell type specificity, and lack of efficient screening and precise analysis methods, engineering of cell wall composition will undoubtedly be the most challenging task of cellulosic feedstock improvement.

Biomass yield is critical for the successful implementation of cellulosic biofuels. C4 plants have the potential to produce exceptionally high grain yield as well as stem and leaf biomass yield (Piedade et al. 1991) and, compared with C3 plants, appear to be more promising as biomass-producing plants for cellulosic biofuels.

C4 plants convert energy more efficiently into biomass than C3 plants and have up to 60% higher water and nutrient use efficiency (Beadle and Long 1985; Beale et al. 1999; Heaton et al. 2008b). By selecting for high-biomass-yielding species, combined with high nutrient and water use efficiency, economically efficient production of biofuel feedstock may be realized on less optimal land without pressuring prime grain crop territories. To thrive in these proposed suboptimal environments, biofuel crops need to be adapted to various stresses such as drought, salt, cold, and low nutrient availability. Criticism of the development of biofuels for ethanol production is often based on a fear of continued displacement of food crops with that of biofuel crops. The expansion of agriculture to provide plant biomass for production of fuels or chemical feedstocks will

require greater use of marginal lands. This will make the production of low per-unit value biomass economical. Perennial crops are essential to bringing marginal lands into sustainable biomass production (Wagoner 1990; Scheinost et al. 2001; Cox et al. 2002), maximizing ecosystem productivity (Field 2001) and minimizing losses of topsoil (Gantzer et al. 1990; Pimentel et al. 1995), water, and nutrients (Randall and Mulla 2001).

Flowering time significantly affects biomass production as the transition from vegetative to reproductive phase negatively impacts biomass accumulation. For the maximum biomass yield of cellulosic biofuel crops, flowering is undesired and, in the case of perennials, plants should senesce only shortly before the end of the vegetation period to relocate nutrients into the rhizome. Manipulating flowering time has long been a breeding goal to enable or prevent flowering at desired times and to increase yield in photoperiod-sensitive crops independent of geographical region (e.g., sorghum) and for other applications. In one biomass crop, sugarcane, flowering alone reduces yields enough to wipe out profit margins in some years (Julien and Soopramanien 1976; Long 1976; Julien et al. 1978; Heinz 1987) and increases disease susceptibility (Ricaud et al. 1980).

Biomass yield is a complex trait and influenced by many plant characteristics. Plant height, tiller number per plant, tiller density, and stem thickness are important biomass yield determinants with complex genetics underlying these traits. Tiller number and density will eventually define the number of plants needed per unit area for optimal biomass production and hence influence the establishment costs for plantations. Depending on the species to be developed, propagation capability (seed, *in vitro* culture, or rhizome), rapid field establishment, and pest and disease resistance are also important traits in cellulosic biofuel crops.

Invasiveness remains a concern for nonnative biofuel crops as well as for native biofuel crops grown outside their normal ecosystems (Rhagu et al. 2006). Many plant species such as corn, rice, wheat, soybean, barley, and sorghum are spectacularly successful as crops grown outside of their natural ranges but show varying degrees of invasiveness ranging from none to high. Invasive characteristics like nodal shoot and aggressive rhizome growth or seed dispersal need to be minimized or eliminated in biofuel crops through the breeding process.

Miscanthus x giganteus, a sterile triploid hybrid of a cross between *Miscanthus sinensis* and *Miscanthus sacchariflorus* (Hodkinson et al. 2002b), has been successfully grown for several years as a biofuel crop in Europe (Clifton-Brown et al. 2001). This particular genotype already combines many of the traits desirable for a biofuel crop. As a perennial C4 plant, it produces consistently high biomass yields over many years with little or no nitrogen application. Rhizome growth is locally contained and

sterility provides protection from outcrossing. However, the yield potential might not be fully used when this variety is cultivated under varying climatic conditions. Furthermore, growing only one single clone holds risk with regard to disease susceptibility. Additionally, nutrient content as well as cell wall composition need to be better amenable to conversion. Early flowering at lower latitudes like the Southern USA currently prevents full realization of its yield potential. Breeding *Miscanthus* genotypes adapted to a wide range of growing conditions with lower lignin and ash content, resulting in more efficient biomass conversion, will further improve its suitability as a biofuel crop. Genetic diversity for *Miscanthus* breeding can be drawn from a large gene pool of different *Miscanthus* species like *M. sinensis*, *M. sacchariflorus*, *M. floridulus*, *Triarrhena lutarioriparia*, and others. Switchgrass (*P. virgatum*) has been utilized as a forage grass and has been tested as a biofuel crop in recent years. However, its present yield is lower than *Miscanthus* (Heaton et al. 2008a) but improved varieties could be a useful addition to the biofuel crop repertoire for sites less suitable for *Miscanthus*. *S. bicolor* is known for its adaptation to several stress conditions, in particular drought, and could complement perennial biofuel crops in establishment years or in arid climates. Breeding perennial sorghum would be of interest as well as interspecific crosses between sorghum and *Miscanthus*. Similarly, Miscane, a hybrid between sugarcane and *Miscanthus*, could potentially combine the high productivity of both species with the perenniality and adaptation of *Miscanthus* to colder climates. Of the main breeding goals, improving cell wall composition and its digestibility is presently deemed most critical for enhancing sugar extraction efficiency and overall economy of cellulosic ethanol production.

Genetic Manipulation to Improve Biofuel Crops

Breeding cellulosic feedstock can initially take advantage of the knowledge and technologies developed for food and forage crops in their long breeding history. This ranges from germplasm collection and characterization to breeding strategy development including marker-assisted selection (MAS) and transgenic modifications. The starting point of a biofuel crop breeding program can vary significantly depending on the extent of genetic improvement. For sorghum and sugarcane, well-established breeding programs have existed for many yr. While the breeding goal has not been for biomass production (e.g., high sugar content versus high biomass for sugarcane), attention to issues such as harvestability and disease resistance may indirectly benefit biofuel breeding. Others, like *Miscanthus* and switchgrass, are essentially “wild” undomesticated plants. The genetic improvement of *Miscanthus* and

switchgrass has to be initiated with the collection of germplasm but at the same time holds much potential for realizing the genetic gain in the first few generations of variety improvement.

Germplasm Collection

Germplasm collections provide breeders with genetic resources for trait improvement in energy crop breeding programs. By cross-pollination and subsequent selection in segregating progenies, plant breeders can reassemble targeted traits in one variety for its optimized performance. Sufficient germplasm variations and genetic information relating to interesting traits are essential for the success of a breeding program.

Looking closely at the available germplasm collections in biofuel crops, such as *S. bicolor*, *Miscanthus*, switchgrass, and Miscane, different pictures emerge for each crop. A large sorghum germplasm collection of >36,000 accessions exists in the USA (Reddy et al. 2006) and at the International Crops Research Institute for the Semi-arid Tropics (Saballo 2008). In contrast, there is practically no public germplasm collection for *Miscanthus*, despite a few collection activities by research institutes, botanical gardens, and private companies who have compiled a small number of landraces and ornamental accessions. Nevertheless, there is rich genetic variation in wild populations of both switchgrass and *Miscanthus*. Switchgrass is native to North America and has been bred as a forage crop. *Miscanthus*, on the other hand, originated in East Asia and nearby Pacific islands. Eleven to 20 species have been identified in *Miscanthus* and ongoing molecular analysis is still being applied to refine our understanding of the phylogenetic relationships between *Miscanthus* species (Hodkinson et al. 2002; Clifton-Brown et al. 2008). The center of diversity for *Miscanthus* is in East China, South Korea, Taiwan, and Japan. In China, limited breeding work has been done in *Miscanthus* to improve its fiber quality and yield as a feedstock for the paper industry (He et al. 1998). Several species of *Saccharum* can be explored for Miscane/energy cane breeding. Sugarcane’s genetic disposition includes several species of the *Saccharum* complex (Dillon et al. 2007) and a large number of collections of *Saccharum* have been maintained in Brazil, India, the USA, and China.

Access to genetic resources is vital to breeders. Because of restricted biomaterial exchange between countries, it is a huge challenge for breeders to access genetic resources if they want to establish a breeding program in a species outside of its natural geographic locations. For example, several obstacles have to be overcome for a breeder in the US to access *Miscanthus* germplasm. While collections of several *Miscanthus* accessions from different locations in China, Japan, Taiwan, and Korea exist, export and import

restrictions and ambiguous regulations under the convention of biological diversity protocol (CBD) complicate the acquisition and transfer of *Miscanthus* germplasm outside these countries for commercial product development. The limited capacity for *Miscanthus* quarantine further delays the germplasm import into the USA. It can take several years before a significant number of *Miscanthus* accessions are available for breeders outside Asia. Similar restrictions have applied to sorghum and sugarcane germplasm exchange since the CBD protocol became effective in 1993.

Germplasm Characterization

Genetic and phenotypic analyses need to be conducted once the germplasm is available to the breeder. Core germplasm accessions should be selected as parents to be used in crossbreeding programs. Of the potential C4 biofuel species, most (except sorghum) are either polyploid or have large complex genomes, complicating genetic analysis. *Miscanthus*, switchgrass, sugarcane, energy cane, big bluestem, Bermuda grass, napier grass, and others have varying ploidy levels from diploid up to octoploid, and their chromosome numbers can be up to 100 or more. *S. bicolor* provides a much needed model for biofuel crop genomics and breeding research. *S. bicolor* is diploid and has a relative small genome of about 730 Mb that has been completely sequenced (Paterson et al. 2009). The sorghum sequence will be a valuable tool for comparative genetic studies. With the sorghum sequence as a reference, syntenic regions or candidate gene sequences can be exploited to identify the locations of genes that govern critical trait inheritance. Subsequently, these syntenic regions or candidate gene sequences can be used to develop markers linked to the traits of interest for marker-assisted selection or cloning of the underlying genes.

Germplasm characterization at the molecular level can shed light onto the genetic diversity and trait inheritance information that are needed by breeders in planning their crosses and subsequent selections. Keeping a degree of genetic diversity in the crossing parents is a common measure to enhance the adaptability of a variety to a broad range of environments. Molecular markers, such as restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), Diversity Arrays Technology (DArT), and single-nucleotide polymorphism (SNP), are frequently used in germplasm characterizations. Each type of marker has somewhat different properties in terms of the nature of polymorphism assayed, the assay methods, equipment requirements, costs, and assay throughput. RFLP, AFLP, and DArT markers do not require sequence information; they can be used for initial genetic characterization of germplasm

collections that have little or no genomic sequences available. DArT markers have been applied in sorghum (Mace et al. 2008), rice (Jaccoud et al. 2001), barley (Wenzl et al. 2004), wheat (Akbari et al. 2006), and others (James et al. 2008). The method has demonstrated good reproducibility and has been applied to higher ploidy levels and consequently large genome size [as demonstrated in hexaploid wheat (Akbari et al. 2006)] and could ultimately be applied for polymorphism analysis in *Miscanthus* and other biofuel candidate crops with higher ploidy levels.

SSR and SNP markers rely on the availability of sequence information for the design of primers or allele-specific oligos. Currently, neither the *Miscanthus* nor the switchgrass genome has been sequenced. Nevertheless, SSRs have become the marker of choice for genetic diversity study and genotype identifications in biofuel crops for the following reasons: (1) large numbers of SSRs have been identified in related species, such as sorghum, sugarcane, corn, wheat, barley, and rice (<http://www.gramene.org/>), and can be used in *Miscanthus* and switchgrass (Hernández et al. 2001). Sugarcane and sorghum are more closely related to *Miscanthus* than corn (Hodkinson et al. 2002) and as such a much higher success rate is expected for sorghum and sugarcane SSRs being validated in *Miscanthus*. (2) SSR polymorphism is derived from numbers of repeats in the flanking sequence and has a high power to differentiate many alleles. (3) SSRs are polymerase-chain-reaction-based markers and thus relatively easy to use. Multiplexing and automation of SSR assays can be performed using capillary-based DNA analyzers in combination with various color fluorescence-labeled primers.

SNPs are powerful for genetic mapping due to their abundance in the genome. Mining of expressed sequence tag libraries is commonly used for SNP discovery (Grivet et al. 2003; Cordeiro et al. 2006; Novaes et al. 2008) and a database for SNP search is available for rice, barley, and brassica (Duran et al. 2009). Conserved intron scanning primers have also been employed for SNP discovery by using conserved locations of introns to design primers followed by identifying sequence variation within the intron (Feltus et al. 2006b). The absence of sufficient genome sequences used to be a bottleneck for SNP discovery application to biofuel crops. However, the new generation of high-throughput DNA sequencing technology such as Illumina (Pavy et al. 2008), 454 (Barbazuk et al. 2007), and SOLiD (Smith et al. 2008) has made SNP discovery rapid, more affordable, and widely available.

Identification of Trait-Linked Markers

Identification of trait-linked molecular markers by genetic mapping is a first step in implementing marker-assisted

selection in breeding improved biomass crops. Draft linkage maps have been published for *M. sinensis* (Atienza et al. 2002) and switchgrass (Missaoui et al. 2005). However, higher densities of molecular markers are needed. Biomass yield and chemical composition are two major goals in biofuel crop breeding. Biotic and abiotic stress tolerance are also important for broad adaptability and yield stability.

Biomass yield of energy crops positively correlates with plant height, stem density, and stem thickness as demonstrated in *Miscanthus* and switchgrass (Atienza et al. 2003; Das et al. 2004; Boe and Beck 2008; Jezowski 2008). In different *Miscanthus* species, plant height can vary from 1.5 to over 5.0 m. Thus, there is large potential for the genetic improvement of plant height in *Miscanthus*. *T. lutarioriparia* is generally tall with thick stems, but stem density is usually low. In contrast, *M. sinensis* is usually short and with stems clumped together at very high density. The stem thickness can vary within a large range, with *M. floridulus* and *M. sacchariflorus* being intermediate types for these traits. In addition to the enormous variations between *Miscanthus* species, the difference between ecotypes of the same species can also be large. Study of the genetic control of the three yield components and subsequently optimizing their value in genetically improved varieties would help breeders achieve their breeding goals for enhanced biomass yield.

Many traits that are high priority for genetic improvement in *Miscanthus* and other biofuel crops are “domestication traits” for which there exists substantial knowledge of genetic control in sorghum and/or other crops and for which the locations of controlling genes/quantitative trait loci (QTLs) often correspond across divergent grasses (Lin et al. 1995; Paterson et al. 1995a, b; Ming et al. 2002; Hu et al. 2003). For example, QTLs controlling tiller number and culm height are known in sorghum (Quinby and Karper 1954; Lin et al. 1995; Hart et al. 2001) and the synteny relationships of plant height QTLs between sorghum and sugarcane have been studied (Ming et al. 2002). Stem diameter itself is a biomass component and also enhances lodging tolerance. A stem diameter QTL which accounted for thicker stems and reduced lodging was identified in rice (Kashiwagi et al. 2008). However, biomass composition and lignin content have to be kept favorable for ethanol extraction with the modification of these traits. Flowering time and photoperiod sensitivity play a critical role in biomass yield through determination of growth period. Delayed or no flowering is desirable for biomass production because an extended vegetative growth period helps produce more biomass and sterility would either reduce or eliminate the invasiveness potential of *Miscanthus* and other biofuel grasses. Several genes and QTLs involved in photoperiod and flowering control have been recognized in sorghum and sugarcane (Quinby and Karper 1945; Rooney and Aydin 1999; Ming et al. 2002), corn, and rice. Similar to

plant height QTLs, QTLs controlling flowering often correspond in sugarcane and sorghum and may be conserved in other Poaceae (Lin et al. 1995; Ming et al. 2002).

The optimal chemical composition of energy crops is determined by the subsequent conversion technologies. Two major conversion technologies are being tested to produce liquid biofuel from plant biomass. One is thermal conversion and the other is bioconversion. These approaches have different requirements for biomass compositions in order to obtain high conversion rates, but both require low ash content. Nutrient recycling of elements such as N, P, and K from the shoots back to roots before harvesting would aid in reducing the ash content. This in turn requires less nutrient uptake by the plant as the nutrients are available from the rhizomes in the following spring. Therefore, reducing ash content not only increases biomass to fuel conversion rate but also reduces overall nutrient uptake. The natural senescence of the plant is essential for nutrient recycling and, in an approach to align maps of two sorghum populations with one common parent, QTLs for leaf senescence could be found (Feltus et al. 2006a). Cell wall composition is cell specific and highly variable depending on the cell type (Nakashima et al. 2008). Parenchyma and collenchyma cells have only primary cell walls whereas sclerenchyma cells have both primary and secondary cell walls. Lignin content is usually higher in secondary cell walls compared to primary cell walls. Studies of altered gene expression in the monolignol biosynthesis pathway in *Arabidopsis* and other dicots as well as in trees revealed changes in both lignin content and composition (Vanholme et al. 2008). Lignin content was negatively correlated with sugar yield in *Miscanthus* (Yoshida et al. 2008). In fact, lignin content could be reduced to a level where untreated transgenic alfalfa yielded higher saccharification efficiency than pretreated control plants (Chen and Dixon 2007). Less information is available on lignin manipulation for grasses; however, the brown midrib, originally a naturally occurring mutant identified in corn, sorghum, and millet, is perhaps the most widely studied trait. While reduced lignin improves saccharification efficiency, it can also reduce biomass yield and survival and also cause lodging (Casler et al. 2002; Vogel et al. 2002; Pedersen et al. 2005; Colemann et al. 2008). However, this seems to be dependent on the genetic background. There are also examples of reduced lignin without obvious phenotypic changes. QTLs for lignin and cell wall components in corn have been successfully identified (Cardinal et al. 2003; Krakowsky et al. 2006; Barrière et al. 2008).

Invasiveness is a concern for novel biofuel crop and can often be contributed to aggressively spreading rhizomes, seed dispersal, or nodal growth. Rhizome growth is generally desired to fill in space between plants but should not be so aggressive that it takes over beyond the field

location. Seed set in biofuel crops is only desired in case of seed production but has not yet been excluded in grasses grown in their native habitats. QTLs for rhizomatousness in rice (Hu et al. 2003) and studies of rhizome growth behavior in *Sorghum propinquum* and *S. bicolor* (Paterson et al. 1995; Jang et al. 2006, 2008) have begun to uncover the genes involved, which are the basis for selection and effective manipulation. Mutations in two genes for seed shattering in rice and a single QTL in sorghum have also been identified (Paterson et al. 1995b; Konishi et al. 2006; Li et al. 2006). The nonshattering gene or QTL-linked markers should be applicable for molecular marker screens in *Miscanthus* or other germplasm. Seed size, seedling vigor, and early cold germination are important traits for fast and successful crop establishment but could also affect invasiveness. These traits need to be balanced and managed in combination with other measures for bioconfinement.

Given the wealth of genetic information for biomass, chemical compositions, and stress tolerance obtained from the study in model plant species such as *Arabidopsis*, rice, sorghum, and corn, a candidate gene approach to identify genes or syntenic regions of interest for equivalent biofuel traits could be effective. This approach relies on the conservation of trait-related synteny/collinearity and gene function between model plants and the biofuel crop of interest. Candidate gene approaches have been successfully demonstrated in plant disease resistance gene mapping and the subsequent cloning (McIntyre et al. 2005). As shown above, many QTLs important for biofuel traits have been identified in sorghum, sugarcane, and rice. The use of conserved markers in genetic mapping should identify related genes or their locations in *Miscanthus* and other biofuel crops.

Traditionally, trait mapping was performed in segregating populations that were derived from biparental crosses. The mapped trait may be restricted by the choice of available genetic resources. Due to the development of high-density SNP markers with a genome-wide coverage, trait-associating mapping becomes feasible using a broad panel of selected genetic resources, such as germplasm accessions, breeding lines, and commercial varieties. The association mapping approach eliminates the necessity of mapping population construction and allows breeders to focus on their most valuable genetic resources. Furthermore, recently high-throughput deep sequencing may yield more closely linked markers or directly identify the gene for the targeted traits without going through tedious genetic mapping procedures (Lister et al. 2009).

Marker-Assisted Selection

Once the genes controlling traits of interest or phenotype-associated molecular markers have been identified, direct

selection for desired genotypes becomes possible through molecular marker analysis. MAS is especially valuable for the improvement of biomass yield, which is complex partially because gene–environment interactions reduce the accuracy of direct phenotype selection. Likewise, diagnostic markers for cell wall composition would help to mitigate the lack of efficient assays for breeders to do rapid assessment (ideally in the field). The implementation of MAS in biofuel crop breeding programs should expedite development of novel cultivars with high biomass and desired chemical compositions.

MAS can significantly improve breeding efficiency by the accurate selection of desired genotypes in early generations or at early growth stages. MAS can also reduce the impact of nongenetic factors that can interfere with phenotype selection and help to quantify the stability of particular genes (or QTLs) across environments. Distinguishing genes that are stable from those that are environment specific may help to tailor genotypes to particular production systems (Saranga et al. 2001; Paterson et al. 2003; Saranga et al. 2004).

Genotype selection by MAS in early generations or at early growth stages is particularly beneficial in biofuel crop breeding programs because most potential biomass crops are perennials and their phenotype expression can be delayed for multiple yr. As an extreme example, application of a marker-assisted backcrossing selection in perennial oil palm would theoretically reduce the breeding cycle from 19 to 13 yr compared to a conventional approach (Wong and Bernardo 2008). Furthermore, biofuel crops tend to be large plants and each plant requires space in greenhouses and breeding nurseries. Plant breeders need to screen thousands of breeding lines or individuals in each generation. A *Miscanthus* plantation reaches its full yield potential in 2 to 3 yr after planting. Any selection that can be practiced by applying diagnostic DNA markers in the first year or even at the seedling stage would significantly save space and time in the breeding process. MAS can be performed in different stages of a breeding program. Marker-assisted germplasm fingerprinting can be used for parent selections. In fact, parental selection based on molecular marker screening has been deemed critical in starting a successful MAS wheat breeding program (Anderson et al. 2007).

MAS has been implemented for both qualitative and quantitative traits in crop breeding programs (Castro et al. 2003; Concibido et al. 2004; Joseph et al. 2004; Schmierer et al. 2005; Zhou et al. 2005; Collard and Mackill 2008; Buerstmayr et al. 2009; Zhou et al. 2009). Major gene-controlled traits can be directly identified by marker analysis and applied for targeted improvement. For example, *Xa21*-mediated disease resistance (Chen et al. 2000) and *Bph1* and *Bph2*-mediated insect resistance (Sharma et

al. 2004) have been identified in elite germplasm collections and introduced into rice cultivars through marker-assisted backcrossing. In the latter case, an additional advantage of MAS has been employed, pyramiding of several traits to maximize phenotype expression.

Marker-assisted gene pyramiding for the development of sustainable disease resistance will become critical in biofuel crop breeding. Large acreage plantings of a vegetative propagated biofuel crop could lead to monoculture of a few selected genotypes, with potentially high vulnerability to disease epidemics. Pyramiding different disease resistance genes against various pathogen strains in elite breeding lines would help reduce genetic vulnerability in released cultivars (Castro et al. 2003). Huang et al. (1997) reported pyramiding of four bacterial blight resistance genes in rice, achieving broad-spectrum disease resistance.

Beyond single-gene traits such as disease resistance, more complex traits such as aroma and root growth have also employed MAS for variety improvement (Steele et al. 2006). Rates of gain in complex (quantitative) trait improvement through MAS depend on the number of QTLs involved and their genetic effects. A range of results have been reported for the improvement of quantitative traits, such as grain yield, by MAS. Bouchez et al. (2002) reported on an extensive MAS program for the improvement of corn elite lines based on QTLs for earliness and grain yield. Testing of the improved varieties showed the expected earliness, but grain yield was negatively impacted. A MAS approach for corn yield under drought stress produced lines that generated higher corn yields under heavy drought stress but these lines lost their superiority over the control lines when the drought stress was less severe (Ribaut and Ragot 2007) and conventional breeding produced the same results (Ribaut and Ragot 2007). Phenotypic screening in both programs was executed only at the very end of the breeding process. As a result, the authors recommend including phenotypic screens during the MAS process. On the other hand, breeding for the quantitatively inherited *Fusarium* head blight resistance could be successfully accomplished by MAS (Anderson et al. 2007). The major QTL (*Fhb1*) produced a consistent phenotype over different genotypes and environments and closely linked markers were available (Anderson et al. 2007; Buerstmayr et al. 2009).

One of the most successful MAS breeding programs to date has been implemented by Monsanto (Eathington et al. 2007; Edgerton 2009). Researchers compared 248 unique soybean populations in a conventional selection with MAS. MAS-derived lines in the Monsanto program were higher performing for a combination of traits including grain yield, but the amount of gains for both methods varied between years. Similar results were reported for sunflower for the improvement of grain yield, oil content, and other traits and

in corn for grain yield and other traits (Eathington et al. 2007). A high genetic gain was observed in early populations. The genetic gain of each selection cycle is very much dependent on the frequency increase of favorable genes. Therefore, marker-assisted genotype evaluation and subsequent selection can apparently help to accumulate desired genotypes in the selected populations.

Although the application of MAS for quantitatively inherited traits is presently more challenging, further development of genetic maps and marker technology will accelerate implementation. Biomass yield, an important target trait in biofuels, is a complex trait and improvement by MAS will pose challenges. Parental selection in a large number of populations seems crucial for selecting the best potential parents as well as the integration of phenotypic selections steps between the marker selection stages (Anderson et al. 2007; Eathington et al. 2007). MAS can be readily applied to biofuels crops once candidate genes have been identified for biomass yield and cell wall composition. MAS will play a significant role in future enhancement of biofuel crops as well as food and feed crops (Edgerton 2009). An even more intriguing approach is the combination of MAS with the transgene technology.

Transgenic Cellulosic Biofuel Crops

Genetically modified crops have successfully been grown for over 10 yr since their debut in 1996. The majority of present transgenic crops comprise just two traits, herbicide resistance and insect resistance, but these have been widely applied to soybean, corn, cotton, and canola. The land cultivated with these crops covered a remarkable 125 million hectares in 2008 (ISAAA 2008: Executive Summary). Since their introduction, transgenic technology and crops have been under scrutiny relative to human health and the environment although transgenic crops which are commercialized are considered generally recognized as safe and approved by various government agencies throughout the world for use in food, feed, and fuel. Gene flow and escape into wild relatives and populations, unintended admixture with nontransgenic crops, and development of herbicide-resistant weeds and plants are concerns that are manageable based on current accepted practices (Council for Agricultural Science and Technology 2007).

The development of cellulosic biofuels feedstock will undoubtedly benefit from transgenic approaches. *Miscanthus*, switchgrass, and other novel crops are obligate outcrossers; hence, gene flow is a potential problem. However, cellulosic nonfood crops have a key advantage over transgenic food crops. Flowering and seed development are undesired. In fact, a cellulosic biofuel crop produces biomass more efficiently if resources are not

directed into flowering. The risk of transgene escape may be dramatically reduced if nonflowering biofuel crops can be produced in the field. The sorghum photoperiod-sensitive gene, e.g. *Ma5* and *Ma6*, involved in flowering of sorghum have been shown to delay flowering significantly when dominantly expressed (Rooney and Aydin 1999). Tobacco plants produced higher biomass although at reduced plant height when an *Arabidopsis* flower repressor (FLC) was expressed causing delayed flowering (Salehi et al. 2005). Additionally, *CENTRORADIALIS* homologs were shown to delay flowering and vastly increased the height of tobacco plants (Amaya et al. 1999). Such findings ultimately provide opportunities to manipulate flowering time in grasses by a transgenic approach. On the other hand, flowering of biofuel crops is desired for recombination in breeding nurseries and crossing fields. Given the restricted areas for breeding purpose, gene escape should be easily manageable by growing crops afar from potential undesired crossing partners. Switchgrass is likely to be grown in its native habitat and a major concern is that transgenic switchgrass could easily hybridize with local population. *S. bicolor*, although self-fertile, can also hybridize with species of the *Saccharum* complex. Ideally, only sterile genotypes would be grown in the field as currently practiced for the sterile *M. x giganteus*. Sterile *Miscanthus* hybrid production is one of the breeding goals which entails the remake of the cross of high-yielding *M. sinensis* with *M. sacchariflorus* to produce a hybrid that outyields *M. x giganteus*. However, due to high propagation costs, the development of high-yielding locally adapted *M. sinensis* and *M. sacchariflorus* which could be sown and produce seeds is an opportunity to reduce establishment costs. Several options for genetic manipulation are available to prevent gene escape (Daniell 2002). Transgenic approaches like male sterility which have been deployed to produce hybrids (Hartley 1988, 1989; Mariani et al. 1991; Li et al. 2007; Gils et al. 2008) or transgene expression in only maternally inherited chloroplasts can reduce risks associated with cross-pollination (Ruf et al. 2001; Wurbs et al. 2007) and are examples for strategies to prevent outcrossing.

Genetically modified food crops are subject to a rigorous screening, testing, and deregulation process. Since biofuel crops are not intended for human or animal consumption, regulatory processes for biofuel crops could become less elaborative and cheaper and potentially accelerate the development and release of transgenic biofuel varieties and reduce costs in the development of novel biofuel crops.

The transfer of foreign DNA into plants has commonly been facilitated by *Agrobacterium*-mediated transformation or particle bombardment for major crops. Of the novel biofuel plants, switchgrass has been transformed with *Agrobacterium* (Somleva et al. 2002) and *M. sacchariflorus*

was reportedly transformed via particle bombardment (Zili et al. 2004). High transformation efficiency protocols for *Agrobacterium*-mediated transformation need to be developed for *Miscanthus* to enable the effective employment of promising genes for crop improvement in further plant generations.

As described above, lignin composition as well as lignin, cellulose, and hemicellulose content most critically determines the efficiency of lignin degradation and saccharification and ultimately affects ethanol yield. Hence, these components are considered main targets of genetic improvement of biofuel feedstock. Transcriptional profiling has only just begun to unravel the complexity of genes regulating the lignin biosynthesis and the effect on genes beyond this pathway. Different levels of lignin and gene expression in various tissues, as well as changes in pathways beyond the lignin pathway, are beginning to illustrate the complexity of lignin biosynthesis. Interestingly, but not surprisingly, an array of transcription factors (TFs) are surfacing as key regulators of secondary cell wall biosynthesis in *Arabidopsis* and trees (Patzlaff et al. 2003; Goicoechea et al. 2005; Zhong et al. 2006; Zhong and Ye 2007; Zhong et al. 2008). TFs of the NAC family appear to be cell-type-specific which activate a cascade of downstream MYB and KNAT TFs which then activate genes of the biosynthetic pathways for cellulose and hemicellulose (Kubo et al. 2005; Mitsuda et al. 2007; Zhong et al. 2008). MYB and LIM TFs have also been suggested to be specifically involved in the lignin pathway (Kawaoka et al. 2000; Patzlaff et al. 2003a, b; Karpinska et al. 2004; Goicoechea et al. 2005; Yang et al. 2007). More studies should identify specific TFs regulating either the lignin or cellulose biosynthesis pathway. Such knowledge can be applied to manipulate particular TFs to optimize secondary cell wall composition. Increased cellulose and hemicellulose content in combination with reduced lignin for enhanced sugar and ethanol yield would produce a particularly valuable cellulosic feedstock. Cell- and tissue-specific gene expression analysis and their interaction in networks will be a trademark of further identification of genes and TFs involved in cell wall biosynthesis.

In addition to lignin and cellulose modification, direct expression of cellulases in plants has been discussed as an alternative way to improve decomposition of plant cell walls and reduce recalcitrance to processing. The complexity of cellulose biosynthesis is expected to require a complex, concerted, and tissue-specific use of enzyme action for cellulose degradation (Taylor et al. 2008). Heterologous expression of glycosyl hydrolases has been used to enhance glucose extraction in rice (Oraby et al. 2007) and corn (Ransom et al. 2007) and could be a strategy for improving overall sugar extraction. The correct expression of these enzymes would be critical to initiate the

cellulose degradation shortly before, at, or immediately after harvest. Suitable promoters or chemical induction methods would be needed to accomplish this step. Another interesting aspect of improving the economic prospects of ethanol production could be to extract higher-value products from the lignin biosynthetic pathway by manipulating this pathway for different by-products and at the same time accomplish to simplify lignin structure for improved degradation (Anderson et al. 2005; Somleva et al. 2008).

Overexpression of transcription factors has been widely demonstrated to enhance resistance to biotic and abiotic stress in model plants as well as in crop plants (Century et al. 2008). Cellulosic biofuel feedstocks will likely be grown on less fertile soil while more fertile soils remain cultivated with food crops. Hence, tolerance to stresses such as drought, salt, cold, and heat will be preferred traits for biofuels. TFs are particularly promising transgenic tools as they regulate not only one gene but complex and often interacting pathways directed toward numerous stress responses. Therefore, changes in gene expression caused by overexpression of a particular TF often provide increased resistance to more than one abiotic stress factor. For example, the NAC family, which also comprises TFs involved in cell wall biosynthesis, has provided cold and salt tolerance when overexpressed in rice and tested under field conditions (Hu et al. 2006). CBF transcription factors of the AP2/ERF family have been demonstrated to provide cold tolerance in *Arabidopsis* (Jaglo-Ottosen et al. 1998; Novillo et al. 2007), as well as orthologs in rice (Ito et al. 2006), corn (Qin et al. 2004), and birch (Welling and Palva 2008) or when AtCBF1 was overexpressed in poplar (Benedict et al. 2006). Moreover, drought and salt tolerance has also been observed in combination with cold tolerance when CBF rice orthologs were constitutively overexpressed in rice (Dubouzet et al. 2003).

Similar to abiotic stress resistance, TFs can also regulate defense responses to pests and diseases. TFs of the ERF subfamily have been demonstrated to enhance disease resistance in *Arabidopsis* and crop plants (Berrocal-Lobo et al. 2002; Guo et al. 2004; Zuo et al. 2007). WRKY TFs have been shown to act at the junction between the jasmonic and the salicylic acid pathways, thereby modifying the crosstalk between these pathways (Li et al. 2004; Mao et al. 2007; Higashi et al. 2008) and defining the plant defense response to pathogen attack.

Breeding for disease resistance in biofuel crops will become imperative with increasing cultivation. Pests and diseases will have a more profound effect on the economy of the biofuel crop if significant damage or total plant loss occurs. *Miscanthus*, switchgrass, and other novel biofuel crops have not yet been grown extensively, but bacterial, viral, and fungal pathogens as well as nematodes have already been reported to attack *Miscanthus* (Christian et al.

1994; O'Neill and Farr 1996; Thinggard 1997; Gams et al. 1999; Halbert and Remaudiere 2000) and switchgrass (Gravert and Munkvold 2002; Gustafson et al. 2003; Krupinski et al. 2004; Carris et al. 2008). Interestingly, *Miscanthus* has been used in sugarcane breeding for improving disease resistance (Chen and Lo 1989; Miller et al. 2005), implicating that there is useful variation for disease resistance in *Miscanthus* germplasm.

A database is now available integrating gene regulatory information for maize, rice, sorghum, and sugarcane (<http://www.grassius.org>, Yilmaz et al. 2009) which could further advance the knowledge of regulatory genes and networks in biofuel grasses and their incorporation into breeding programs for improved genotypes. Gene stacking and the use of tissue-specific or inducible promoters for precise expression will be needed for the genetic engineering of biofuel crops because of the interaction networks of transcription factors and the potential need to add multiple traits. Transgenic corn with more than one introduced trait (one for glyphosate herbicide resistance and the other for resistance for the European corn borer and corn rootworm) is currently in commercial use (Dill et al. 2008) and shows no adverse effects on forage quality (McCann et al. 2007; Drury et al. 2008). New strategies for pyramiding several genes or integrating gene regions into plants via mini-chromosomes in corn are also being developed (Carlson et al. 2007) and could be beneficial for biofuel crop advancement.

Cell wall degradability, biomass yield, and stress resistance are the major traits which will ultimately define the suitability and use of cellulosic biofuels. Optimizing these traits through transgene technology can be considered a promising tool for effectively developing cellulosic biofuel crops. Given that flowering is an undesired trait in biofuel crops, gene flow could much more easily be avoided by eliminating or altering flowering. In addition, deregulation of transgenic biofuel crops should be more straightforward because biofuels are not used for human or animal consumption. In summary, applying transgenic technology to biofuel crops has fewer hurdles than transgenic food crops and should enable much faster variety development.

Conclusion

The potential for significant genetic improvement of C4 grasses as biofuel crops is good. Full exploration of natural genetic resources through plant breeding with the aid of molecular tools could dramatically increase biomass yield of dedicated biofuel crops and thus meet the demand of feedstocks for biofuel production without a significant impact on our food supply and natural environment.

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References

- Akbari M.; Wenzl P.; Caig V.; Carling J.; Xia L.; Yang S.; Uszynski G.; Mohler V.; Lehmensiek A.; Kuchel H.; Hayden M. J.; Howes N.; Sharp P.; Vaughan P.; Rathmell B.; Huttner H.; Kilian A. Diversity arrays technology (DArT) for high-throughput profiling of the hexaploid wheat genome. *Theor. Appl. Genet* 113: 1409–1420; 2006. doi:10.1007/s00122-006-0365-4.
- Amaya I.; Ratcliffe O. J.; Bradley D. J. Expression of *CENTRORADIALIS* (*CEN*) and *CEN*-like genes in tobacco reveals a conserved mechanism controlling phase change in diverse species. *Plant. Cell* 11: 1405–1417; 1999.
- Anderson J. A.; Chao S.; Liu S. Molecular breeding using a major QTL for *Fusarium* head blight resistance in wheat. *Crop Sci* 47: S112–S119; 2007. doi:10.2135/cropsci2006.05.0359.
- Anderson W. F.; Peterson J.; Akin D. E.; Morrison W. H. Enzyme pretreatment of grass lignocellulose for potential high-value co-products and an improved fermentable substrate. *Appl. Biochem. Biotechnol* 121: 303–310; 2005 doi:10.1385/ABAB:121:1-3:0303.
- Atienza S. G.; Satovic Z.; Petersen K. K.; Dolstra O.; Martín A. Preliminary genetic linkage map of *Miscanthus sinensis* with RAPD markers. *Theor. Appl. Genet* 105: 946–952; 2002 doi:10.1007/s00122-002-0956-7.
- Atienza S. G.; Satovic Z.; Petersen K. K.; Dolstra O.; Martín A. Identification of QTLs influencing agronomic traits in *Miscanthus sinensis* Anders. I. Total height, flag-leaf height and stem diameter. *Theor. Appl. Genet* 107: 123–129; 2003 doi:10.1007/s00122-003-1218-z.
- Barbazuk W. B.; Emrich S. J.; Chen H. D.; Li L.; Schnable P. S. SNP discovery via 454 transcriptome sequencing. *Plant. J* 51: 910–918; 2007 doi:10.1111/j.1365-313X.2007.03193.x.
- Barrière Y.; Ralph J.; Méchin V.; Guillaumie S.; Grabber J. H.; Argillier O.; Chabbert B.; Lapiere C. Genetic and molecular basis of grass cell wall biosynthesis and degradability. II. Lessons from brown-midrib mutants. *C. R. Biol* 327: 847–860; 2004 doi:10.1016/j.crvi.2004.05.010.
- Barrière Y.; Thomas J.; Denoue D. QTL mapping for lignin content, lignin monomeric composition, *p*-hydroxycinnamate content, and cell wall digestibility in the maize recombinant inbred line progeny F838 × F286. *Plant Sci* 175: 585–595; 2008 doi:10.1016/j.plantsci.2008.06.009.
- Baucher M.; Bernard-Vailhé M. A.; Chabbert B.; Besle J. M.; Opsomer C.; Van Montagu M.; Botterman J. Down-regulation of cinnamyl alcohol dehydrogenase in transgenic alfalfa (*Medicago sativa* L.) and the effect on lignin composition and digestibility. *Plant Mol. Biol* 39: 437–447; 1999 doi:10.1023/A:1006182925584.
- Baucher M.; Halpin C.; Petit-Conil M.; Boerjan W. Lignin: genetic engineering and impact on pulping. *Crit. Rev. Biochem. Mol. Biol* 38: 305–350; 2003 doi:10.1080/10409230391036757.
- Beadle C. L.; Long S. P. Photosynthesis—is it limiting to biomass production? *Biomass* 8: 119–168; 1985 doi:10.1016/0144-4565(85)90022-8.
- Beale C. V.; Morison J. I. L.; Long S. P. Water use efficiency of C-4 perennial grasses in a temperate climate. *Agric. Forest Meteorol* 96: 103–115; 1999 doi:10.1016/S0168-1923(99)00042-8.
- Benedict C.; Skinner J. S.; Meng R.; Chang Y.; Bhalerao R.; Huner N. P.; Finn C. E.; Chen T. H.; Hurry V. The CBF1-dependent low temperature signaling pathway, regulon and increase in freeze tolerance are conserved in *Populus* spp. *Plant Cell Environ* 29: 1259–1272; 2006 doi:10.1111/j.1365-3040.2006.01505.x.
- Berrocal-Lobo M.; Molina A.; Solano R. Constitutive expression of ETHYLENE-RESPONSE-FACTOR1 in *Arabidopsis* confers resistance to several necrotrophic fungi. *Plant J* 29: 23–32; 2002 doi:10.1046/j.1365-313x.2002.01191.x.
- Boe A.; Beck D. L. Yield components of biomass in switchgrass. *Crop. Sci* 48: 1306–1311; 2008 doi:10.2135/cropsci2007.08.0482.
- Bouchez A.; Hospital F.; Causse M.; Gallais A.; Charcosset A. Marker-assisted introgression of favorable alleles at quantitative trait loci between maize elite lines. *Genetics* 162: 1945–1959; 2002.
- Buerstmayr H.; Ban T.; Anderson J. A. QTL mapping and marker-assisted selection for *Fusarium* head blight resistance in wheat: a review. *Plant Breed* 128: 1–26; 2009 doi:10.1111/j.1439-0523.2008.01550.x.
- Cardinal A. J.; Lee M.; Moore K. J. Genetic mapping and analysis of quantitative trait loci affecting fiber and lignin content in maize. *Theor. Appl. Genet* 106: 866–874; 2003.
- Carlson S. R.; Rudgers G. W.; Zieler H.; Mach J. M.; Luo S.; Grunden E.; Krol C.; Copenhaver G. P.; Preuss D. Meiotic transmission of an *in vitro*-assembled autonomous maize minichromosome. *Publ. Libr. Sci. Genetics* 310: e179; 2007.
- Carris L. M.; Castlebury L. A.; Zale J. First report of *Tilletia pulcherrima* bunt on switchgrass (*Panicum virgatum*) in Texas. *Plant Dis* 92: 1707; 2008 doi:10.1094/PDIS-92-12-1707C.
- Casler M. D.; Buxton D. R.; Vogel K. P. Genetic modification of lignin concentration affects fitness of perennial herbaceous plants. *Theor. Appl. Genet* 104: 127–131; 2002 doi:10.1007/s001220200015.
- Castro A. J.; Capettini F.; Corey A. E.; Filichkina T.; Hayes P. M.; Kleinhofs A.; Kudrna D.; Richardson K.; Sandoval-Islas S.; Rossi C.; Vivar H. Mapping and pyramiding of qualitative and quantitative resistance to stripe rust in barley. *Theor. Appl. Genet* 107: 922–930; 2003 doi:10.1007/s00122-003-1329-6.
- Century K.; Reuber T. L.; Ratcliffe O. J. Regulating the regulators: the future prospects for transcription-factor-based agricultural biotechnology products. *Plant Physiol* 147: 20–29; 2008 doi:10.1104/pp.108.117887.
- Chen F.; Dixon R. A. Lignin modification improves fermentable sugar yields for biofuel production. *Nat. Biotechnol* 25: 759–761; 2007 doi:10.1038/nbt1316.
- Chen S.; Lin X. H.; Xu C. G.; Zhang Q. Improvement of bacterial blight resistance of ‘Minghui 63’, an elite restorer line of hybrid rice, by molecular marker-assisted selection. *Crop Sci* 40: 239–244; 2000.
- Chen Y. H.; Lo C. C. Disease resistance and sugar content in *Saccharum*–*Miscanthus* hybrids. *Taiwan Sugar* 36: 9–12; 1989.
- Christian D. G.; Lamphey J. N. L.; Forde S. M. D.; Plumb R. T. First report of barley yellow dwarf luteovirus on *Miscanthus* in the United Kingdom. *Eur. J. Plant Pathol* 100: 167–170; 1994 doi:10.1007/BF01876249.
- Clifton-Brown J.; Chiang Y-C.; Hodkinson T. *Miscanthus*: genetic resources and breeding potential to enhance bioenergy production. In: Vermerris W. (ed) Genetic improvement of bioenergy crops. Springer, New York, pp 273–294; 2008.
- Clifton-Brown J. C.; Lewandowski I.; Andersson B.; Basch G.; Christian D. G.; Bonderup-Kjeldsen J.; Jørgensen U.; Mortensen V.; Riche A. B.; Schwarz K. U.; Tayebi K.; Teixeira F. Performance of 15 *Miscanthus* genotypes at five sites in Europe. *Agron. J* 93: 1013–1019; 2001.
- Coleman H. D.; Samuels A. L.; Guy R. D.; Mansfield S. D. Perturbed lignification impacts tree growth in hybrid poplar—a function of sink strength; vascular integrity; and photosynthetic assimilation. *Plant Physiol* 148: 1229–1237; 2008 doi:10.1104/pp.108.125500.
- Collard B. C. Y.; Mackill D. J. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Phil. Trans. R. Soc. B* 363: 557–572; 2008 doi:10.1098/rstb.2007.2170.

- Concibido V. C.; Diers B. W.; Arelli P. R. A decade of QTL mapping for cyst nematode resistance in soybean. *Crop Sci* 44: 1121–1131; 2004.
- Council for Agricultural Science and Technology (CAST) Implications of gene flow in the scale-up and commercial use of biotechnology-derived crops: economic and policy considerations. issue paper 37. CAST, Ames, Iowa; 2007.
- Cordeiro G. M.; Elliott F.; McIntyre C. L.; Casu R. E.; Henry R. J. Characterization of single nucleotide polymorphisms in sugarcane ESTs. *Theor. Appl. Genet* 113: 331–343; 2006 doi:10.1007/s00122-006-0300-8.
- Cox T. S.; Bender M.; Picone C.; Van Tassel D. L.; Holland J. B.; Brummer E. C.; Zoeller B. E.; Paterson A. H.; Jackson W. Breeding perennial grain crops. *Crit. Rev. Plant Sci* 21: 59–91; 2002 doi:10.1080/0735-260291044188.
- Daniell H. Molecular strategies for gene containment in transgenic crops. *Nature Biotechnol.* 20: 581–586; 2002 doi:10.1038/nbt0602-581.
- Das M. K.; Fuentes R. G.; Taliaferro C. M. Genetic variability and trait relationships in switchgrass. *Crop Sci* 44: 443–448; 2004.
- Davison B. H.; Drescher S. R.; Tuskan G. A.; Davis M. F.; Nghiem N. P. Variation of S/G ratio and lignin content in a *Populus* family influences the release of xylose by dilute acid hydrolysis. *Appl. Biochem. Biotechnol* 130: 427–435; 2006 doi:10.1385/ABAB:130:1:427.
- DeBolt S.; Gutierrez R.; Ehrhardt D. W.; Melo C. V.; Ross L.; Cutler S. R.; Somerville C.; Bonetta D. Morlin; an inhibitor of cortical microtubule dynamics and cellulose synthase movement. *Proc. Natl. Acad. Sci. U. S. A.* 104: 5854–5859; 2007 doi:10.1073/pnas.0700789104.
- Desprez T.; Juraniec M.; Crowell E. F.; Jouy H.; Pochylova Z.; Parcy F.; Höfte H.; Gonneau M.; Vernhettes S. Organization of cellulose synthase complexes involved in primary cell wall synthesis in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. U. S. A.* 104: 15572–15577; 2007 doi:10.1073/pnas.0706569104.
- Dill G. M.; Cajacob C. A.; Padgett S. R. Glyphosate-resistant crops: adoption; use and future considerations. *Pest. Manag. Sci* 64: 326–331; 2008 doi:10.1002/ps.1501.
- Dillon S. L.; Shapter F. M.; Henry R. J.; Cordeiro G.; Izquierdo L.; Lee L. S. Domestication to crop improvement: genetic resources for *Sorghum* and *Saccharum* (Andropogoneae). *Ann. Bot (Lond)* 100: 975–989; 2007 doi:10.1093/aob/mcm192.
- Drury S. M.; Reynolds T. L.; Ridley W. P.; Bogdanova N.; Riordan S.; Nemeth M. A.; Sorbet R.; Trujillo W. A.; Breeze M. L. Composition of forage and grain from second-generation insect-protected corn MON 89034 is equivalent to that of conventional corn (*Zea mays* L.). *J. Agric. Food Chem* 56: 4623–4630; 2008 doi:10.1021/jf800011u.
- Dubouzet J. G.; Sakuma Y.; Ito Y.; Kasuga M.; Dubouzet E. G.; Miura S.; Seki M.; Shinozaki K.; Yamaguchi-Shinozaki K. OsDREB genes in rice; *Oryza sativa* L.; encode transcription activators that function in drought-, high-salt- and cold-responsive gene expression. *Plant J* 33: 751–763; 2003 doi:10.1046/j.1365-313X.2003.01661.x.
- Duran C.; Appleby N.; Clark T.; Wood D.; Imelfort M.; Batley J.; Edwards D. AutoSNPdb: an annotated single nucleotide polymorphism database for crop plants. *Nucleic Acids Res.* 37: D951–D953; 2009.
- Eathington S. R.; Crosbie T. M.; Edwards M. D.; Reiter R. S.; Bull J. K. Molecular markers in a commercial breeding program. *Crop Sci* 47: S154–S163; 2007 doi:10.2135/cropsci2007.04.0015IPBS.
- Edgerton M. D. Increasing crop productivity to meet global needs for feed; food; and fuel. *Plant Physiol* 149: 7–13; 2009 doi:10.1104/pp.108.130195.
- Feltus F. A.; Hart G. E.; Schertz K. F.; Casa A. M.; Kresovich S.; Abraham S.; Klein P. E.; Brown P. J.; Paterson A. H. Alignment of genetic maps and QTLs between inter- and intra-specific sorghum populations. *Theor. Appl. Genet* 112: 1295–305; 2006a doi:10.1007/s00122-006-0232-3.
- Feltus F. A.; Singh H. P.; Lohithaswa H. C.; Schulze S. R.; Silva T. D.; Paterson A. H. A comparative genomics strategy for targeted discovery of single-nucleotide polymorphisms and conserved-noncoding sequences in orphan crops. *Plant Physiol* 140: 1183–1191; 2006b doi:10.1104/pp.105.074203.
- Field C. B. Sharing the garden. *Science* 294: 2490–2491; 2001 doi:10.1126/science.1066317.
- Gams W.; Klammer M.; O'Donnell K. *Fusarium miscanthi* sp. nov. from *Miscanthus* litter. *Mycol* 91: 263–268; 1999 doi:10.2307/3761371.
- Gantzer C. J.; Anderson S. H.; Thompson A. L.; Brown J. R. Estimating soil erosion after 100 years of cropping on Sanborn Field. *J. Soil Water Conserv* 45: 641–644; 1990.
- Gils M.; Marillonnet S.; Werner S.; Grützner R.; Giritch A.; Engler C.; Schachschneider R.; Klimyuk V.; Gleba Y. A novel hybrid seed system for plants. *Plant Biotechnol. J* 6: 226–235; 2008 doi:10.1111/j.1467-7652.2007.00318.x.
- Goicoechea M.; Lacombe E.; Legay S.; Mihaljevic S.; Rech P.; Jauneau A.; Lapiere C.; Pollet B.; Verhaegen D.; Chaubet-Gigot N.; Grima-Pettenati J. EgMYB2; a new transcriptional activator from *Eucalyptus* xylem; regulates secondary cell wall formation and lignin biosynthesis. *Plant J* 43: 553–567; 2005 doi:10.1111/j.1365-313X.2005.02480.x.
- Gravert C. E.; Munkvold G. P. Fungi and diseases associated with cultivated switchgrass in Iowa. *J. Iowa Acad Sci* 109: 30–34; 2002.
- Grivet L.; Glaszmann J. C.; Vincentz M.; da Silva F.; Arruda P. ESTs as a source for sequence polymorphism discovery in sugarcane: example of the *Adh* genes. *Theor. Appl. Genet* 106: 190–197; 2003.
- Guo Z. J.; Chen X. J.; Wu X. L.; Ling J. Q.; Xu P. Overexpression of the AP2/ EREBP transcription factor OPBP1 enhances disease resistance and salt tolerance in tobacco. *Plant Mol. Biol* 55: 607–618; 2004 doi:10.1007/s11103-004-1521-3.
- Gustafson D. M.; Boe A.; Jin Y. Genetic variation for *Puccinia emaculata* infection in switchgrass. *Crop Sci* 43: 755–759; 2003.
- Halbert S. E.; Remaudiere G. A new oriental *Melanaphis* species recently introduced in North America [Hemiptera; Aphididae]. *Rev. Fr. Entomol.* 22: 109–117; 2000.
- Hart G. E.; Schertz K. F.; Peng Y.; Syed N. H. Genetic mapping of *Sorghum bicolor* (L.) Moench QTLs that control variation in tillering and other morphological characters. *Theor. Appl. Genet* 103: 1232–1242; 2001 doi:10.1007/s001220100582.
- Hartley R. W. Bamase and barstar: expression of its cloned inhibitor permits expression of a cloned ribonuclease. *J. Mol. Biol* 202: 913–915; 1988 doi:10.1016/0022-2836(88)90568-2.
- Hartley R. W. Bamase and barstar: two small proteins to fold and fit together. *Trends Biochem. Sci* 14: 450–454; 1989 doi:10.1016/0968-0004(89)90104-7.
- He L. Z.; Zhou P. H.; Liu X. M.; Cao X. J.; Cao M. D.; Liu Y. S. Studies on the autotetraploid of *Triarrhena lutarioriparia* L. Liou sp. nov. *Acta. Genetica. Sinica* 25: 49–55; 1998.
- Heaton E. A.; Dohleman F. G.; Long S. P. Meeting US biofuel goals with less land: the potential of *Miscanthus*. *Global Change Biol* 14: 2000–2014; 2008a doi:10.1111/j.1365-2486.2008.01662.x.
- Heaton E. A.; Mascia P.; Flavell R.; Thomas S.; Long P. S.; Dohleman F. G. Energy crop development: current progress and future prospects. *Curr. Opin. Biotechnol* 19: 202–209; 2008b doi:10.1016/j.copbio.2008.05.001.
- Heinz D. Sugarcane improvement through breeding. Elsevier, Amsterdam 1987.
- Hernández P.; Dorado G.; Laurie D. A.; Martín A.; Snape J. W. Microsatellites and RFLP probes from maize are efficient sources

- of molecular markers for the biomass energy crop *Miscanthus*. *Theor. Appl. Genet.* 102:616–622; 2001.
- Higashi K.; Ishiga Y.; Inagaki Y.; Toyoda K.; Shiraishi T.; Ichinose Y. Modulation of defense signal transduction by flagellin-induced WRKY41 transcription factor in *Arabidopsis thaliana*. *Mol. Genet. Genom* 279: 303–312; 2008 doi:10.1007/s00438-007-0315-0.
- Hodkinson T. R.; Chase M. W.; Lledó M. D.; Salamin N.; Renvoize S. A. Phylogenetics of *Miscanthus*; *Saccharum* and related genera (Saccharinae; Andropogoneae; Poaceae) based on DNA sequences from ITS nuclear ribosomal DNA and plastid trnL intron and trnL-F intergenic spacers. *J. Plant Res* 115: 381–392; 2002a doi:10.1007/s10265-002-0049-3.
- Hodkinson T. R.; Chase M. W.; Takahashi C.; Leitch I. J.; Bennett M. D.; Renvoize S. A. The use of DNA sequencing (ITS and trnL-F); AFLP; and fluorescent *in situ* hybridization to study allopolyploid *Miscanthus* (Poaceae). *Amer. J. Bot.* 89: 279–286; 2002b doi:10.3732/ajb.89.2.279.
- Hu F. Y.; Tao D. Y.; Sacks E.; Fu B. Y.; Xu P.; Li J.; Yang Y.; McNally K.; Khush K. S.; Paterson A. H.; Li Z.-K. Convergent evolution of perenniality in rice and sorghum. *Proc. Natl. Acad. Sci. U. S. A.* 100: 4050–4054; 2003 doi:10.1073/pnas.0630531100.
- Hu H.; Dai M.; Yao J.; Xiao B.; Li X.; Zhang Q.; Xiong L. Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. *Proc. Natl. Acad. Sci. U. S. A.* 103: 12987–12992; 2006.
- Hu H.; You J.; Fang Y.; Zhu X.; Qi Z.; Xiong L. Characterization of transcription factor gene SNAC2 conferring cold and salt tolerance in rice. *Plant Mol. Biol* 67: 169–181; 2008 doi:10.1007/s11103-008-9309-5.
- Hu W.-J.; Harding S. A.; Lung J.; Popko J. L.; Ralph J.; Stokke D. D.; Tsai C.-J.; Chiang V. L. Repression of lignin biosynthesis promotes cellulose accumulation and growth in transgenic trees. *Nature Biotech* 17: 808–812; 1999 doi:10.1038/11758.
- Huang N.; Angeles E. R.; Domingo J.; Magpantay G.; Singh S.; Zhang G.; Kumaravel N.; Bennett J.; Khush G. S. Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using RFLP and PCR. *Theor. Appl. Genet* 95: 313–320; 1997 doi:10.1007/s001220050565.
- ISAAA. Brief 38-2008: Executive summary. Global status of commercialized biotech/GM crops: 2008 the first thirteen years; 1996 to 2008. <http://www.isaaa.org/resources/publications/briefs/39/executivesummary/default.html>; 2008.
- Ito Y.; Katsura K.; Maruyama K.; Taji T.; Kobayashi M.; Seki M.; Shinozaki K.; Yamaguchi-Shinozaki K. Functional analysis of rice DREB1/CBF-type transcription factors involved in cold-responsive gene expression in transgenic rice. *Plant Cell Physiol.* 47: 141–153; 2006 doi:10.1093/pcp/pci230.
- Jaccoud D.; Peng K.; Feinstein D.; Kilian A. Diversity arrays: a solid state technology for sequence information independent genotyping. *Nucleic Acids Res.* 29: E25; 2001 doi:10.1093/nar/29.4.e25.
- Jaglo-Ottosen K. R.; Gilmour S. J.; Zarka D. G.; Schabenberger O.; Thomashow M. F. Arabidopsis CBF1 overexpression induces cor genes and enhances freezing tolerance. *Science* 280: 104–106; 1998 doi:10.1126/science.280.5360.104.
- James K. E.; Schneider H.; Ansell S. W.; Evers M.; Robba L.; Cuszynski G.; Pedersen N.; Newton A. E.; Russell S. J.; Vogel J. C.; Kilian A. Diversity arrays technology (DArT) for pan-genomic evolutionary studies of non-model organisms. *Publ. Libr. Sci. ONE* 32: e1682; 2008.
- Jang C. S.; Kamps T. L.; Skinner D. N.; Schulze S. R.; Vencill W. K.; Paterson A. H. Functional classification; genomic organization; putatively cis-acting regulatory elements; and relationship to quantitative trait loci; of sorghum genes with rhizome-enriched expression. *Plant Physiol* 142: 1148–1159; 2006 doi:10.1104/pp.106.082891.
- Jang C. S.; Kamps T. L.; Tang H.; Bowers J. E.; Lemke C.; Paterson A. H. Evolutionary fate of rhizome-specific genes in a non-rhizomatous *Sorghum* genotype. *Heredity* 102: 266–273; 2008 doi:10.1038/hdy.2008.119.
- Jeżowski S. Yield traits of six clones of *Miscanthus* in the first 3 years following planting in Poland. *Ind. Crop Prod* 27: 65–68; 2008 doi:10.1016/j.indcrop.2007.07.013.
- Joseph M.; Gopalakrishnan S.; Sharma R. K.; Singh V. P.; Singh A. K.; Singh N. K.; Mohapatra T. Combining bacterial blight resistance and Basmati quality characteristics by phenotypic and molecular marker-assisted selection in rice. *Mol. Breed* 13: 377–387; 2004 doi:10.1023/B:MOLB.0000034093.63593.4c.
- Julien M.; Delaveau P.; Soopramanien G.; Martine J. Age; time of harvest; and environment as factors influencing differences in yield between flowering and vegetative canes. *Proc. Int. Soc. Sugarcane Technol* 16: 1771–1789; 1978.
- Julien M.; Soopramanien G. The effect of flowering on yield in sugarcane. *Rev. Agric. Sucri. Ile Maurice* 55: 151–158; 1976.
- Jung H. G.; Vogel K. P. Influence of lignin on digestibility of forage cell wall material. *J. Anim. Sci.* 62: 1703–1712; 1986.
- Karpinska B.; Karlsson M.; Srivastava M.; Stenberg A.; Schrader J.; Sterky F.; Bhalerao R.; Wingsle G. MYB transcription factors are differentially expressed and regulated during secondary vascular tissue development in hybrid aspen. *Plant Mol. Biol* 56: 255–270; 2004 doi:10.1007/s11103-004-3354-5.
- Kashiwagi T.; Togawa E.; Hirotsu N.; Ishimaru K. Improvement of lodging resistance with QTLs for stem diameter in rice (*Oryza sativa* L.). *Theor. Appl. Genet* 117: 749–757; 2008 doi:10.1007/s00122-008-0816-1.
- Kawaoka A.; Kaothien P.; Yoshida K.; Endo S.; Yamada K.; Ebinuma H. Functional analysis of tobacco LIM protein Ntlm1 involved in lignin biosynthesis. *Plant J* 22: 289–301; 2000 doi:10.1046/j.1365-3113x.2000.00737.x.
- Kelley S. S.; Rowell R. M.; Davis M.; Jurich C. K.; Ibach R. Rapid analysis of the chemical composition of agricultural fibers using near infrared spectroscopy and pyrolysis molecular beam mass spectrometry. *Biomass Bioenergy* 27: 77–88; 2004 doi:10.1016/j.biombioe.2003.11.005.
- Konishi S.; Izawa T.; Lin S. Y.; Ebana K.; Fukuta Y.; Sasaki T.; Yano M. An SNP caused loss of seed shattering during rice domestication. *Science* 312: 1392–1396; 2006 doi:10.1126/science.1126410.
- Krakowsky M. D.; Lee M.; Coors J. G. Quantitative trait loci for cell wall components in recombinant inbred lines of maize (*Zea mays* L.) II: leaf sheath tissue. *Theor. Appl. Genet* 112: 717–726; 2006 doi:10.1007/s00122-005-0175-0.
- Krupinsky J. M.; Berdahl J. D.; Schoch C. L.; Rossman A. Y. A new leaf spot disease on switchgrass (*Panicum virgatum*) caused by *Bipolaris oryzae*. *Can. J. Plant Pathol* 26: 371–378; 2004.
- Kubo M.; Udagawa M.; Nishikubo N.; Horiguchi G.; Yamaguchi M.; Ito J.; Mimura T.; Fukuda H.; Demura T. Transcription switches for protoxylem and metaxylem vessel formation. *Genes Dev* 19: 1855–1860; 2005 doi:10.1101/gad.1331305.
- Labbé N.; Ye P. X.; Franklin J. A.; Womac A. R.; Tyler D. D.; Rials T. G. Analysis of switchgrass characteristics using near infrared techniques. *BioRes* 3: 1329–1348; 2008.
- Li C.; Zhou A.; Sang T. Rice domestication by reducing shattering. *Science* 311: 1936–1939; 2006 doi:10.1126/science.1123604.
- Li J.; Brader G.; Palva E. T. The WRKY70 transcription factor: a node of convergence for jasmonate-mediated and salicylate-mediated signals in plant defense. *Plant Cell* 16: 319–331; 2004 doi:10.1105/tpc.016980.
- Li L.; Zhou Y.; Cheng X.; Sun J.; Marita J. M.; Ralph J.; Chiang V. L. Combinatorial modification of multiple lignin traits in trees through multigene cotransformation. *Proc. Natl. Acad. Sci. U. S. A.* 100: 4939–4944; 2003 doi:10.1073/pnas.0831166100.

- Li S. F.; Iacuone S.; Parish R. W. Suppression and restoration of male fertility using a transcription factor. *Plant Biotechnol. J* 5: 297–312; 2007 doi:10.1111/j.1467-7652.2007.00242.x.
- Lin Y. R.; Schertz K. F.; Paterson A. H. Comparative analysis of QTLs affecting plant height and maturity across the Poaceae; in reference to an interspecific sorghum population. *Genetics* 141: 391–411; 1995.
- Lindeboom J.; Mulder B. M.; Vos J. W.; Ketelaar T.; Emons A. M. Cellulose microfibril deposition: coordinated activity at the plant plasma membrane. *J. Microsc* 231: 192–200; 2008 doi:10.1111/j.1365-2818.2008.02035.x.
- Lister R.; Gregory B. D.; Ecker J. R. Next is now: new technologies for sequencing of genomes; transcriptomes; and beyond. *Curr. Opin. Plant Biol* 12: 1–12; 2009 doi:10.1016/j.pbi.2008.12.005.
- Long A. A large varietal difference in cane deterioration due to flowering. *Pro. South Afr. Sugar Technol. Assoc* 50: 78–81; 1976.
- Mace E. S.; Xia L.; Jordan D. R.; Halloran K.; Parh D. K.; Huttner E.; Wenzl P.; Kilian A. DArT markers: diversity analyses and mapping in *Sorghum bicolor*. *BMC Genomics* 9: 26; 2008 doi:10.1186/1471-2164-9-26.
- Mao P.; Duan M.; Wei C.; Li Y. WRKY62 transcription factor acts downstream of cytosolic NPR1 and negatively regulates jasmonate-responsive gene expression. *Plant Cell Physiol* 48: 833–842; 2007 doi:10.1093/pcp/pcm058.
- Mariani C.; Goldberg R. B.; Leemans J. Engineered male sterility in plants. *Symp. Soc. Exp. Biol* 45: 271–279; 1991.
- McCann M. C.; Carpita N. C. Designing the deconstruction of plant cell walls. *Curr. Opin. Plant Biol* 11: 314–320; 2008 doi:10.1016/j.pbi.2008.04.001.
- McCann M. C.; Trujillo W. A.; Riordan S. G.; Sorbet R.; Bogdanova N. N.; Sidhu R. S. Comparison of the forage and grain composition from insect-protected and glyphosate-tolerant MON 88017 corn to conventional corn (*Zea mays* L.). *J. Agric. Food Chem* 16: 4034–4042; 2007 doi:10.1021/jf063499a.
- McIntyre C. L.; Casu R. E.; Drenth J.; Knight D.; Whan V. A.; Croft B. J.; Jordan D. R.; Manners J. M. Resistance gene analogues in sugarcane and sorghum and their association with quantitative trait loci for rust resistance. *Genome* 48: 391–400; 2005 doi:10.1139/g05-006.
- Miller J. D.; Tai P. Y.; Edme S. J.; Comstock J. C.; Glaz B. S.; Gilbert R. A. Basic germplasm utilization in the sugarcane development program at Canal Point; FL; USA. *Int. Soc. Sugar Cane Technol. Proc.* 2: 532–536; 2005.
- Ming R.; Del Monte T. A.; Hernandez E.; Moore P. H.; Irvine J. E.; Paterson A. H. Comparative analysis of QTLs affecting plant height and flowering among closely-related diploid and polyploid genomes. *Genome* 45: 794–803; 2002 doi:10.1139/g02-042.
- Missaoui A. M.; Paterson A. H.; Bouton J. H. Investigation of genomic organization in switchgrass (*Panicum virgatum* L.) using DNA markers. *Theor. Appl. Genet* 110: 1372–1383; 2005 doi:10.1007/s00122-005-1935-6.
- Mitsuda N.; Iwase A.; Yamamoto H.; Yoshida M.; Seki M.; Shinozaki K.; Ohme-Takagi M. NAC transcription factors; NST1 and NST3; are key regulators of the formation of secondary walls in woody tissues of *Arabidopsis*. *Plant Cell* 19: 270–280; 2007 doi:10.1105/tpc.106.047043.
- Mitsuda N.; Seki M.; Shinozaki K.; Ohme-Takagi M. The NAC transcription factors NST1 and NST2 of *Arabidopsis* regulate secondary wall thickening and are required for anther dehiscence. *Plant Cell* 17: 2993–3006; 2005 doi:10.1105/tpc.105.036004.
- Nakashima J.; Chen F.; Jackson L.; Shadle G.; Dixon R. A. Multi-site genetic modification of monolignol biosynthesis in alfalfa (*Medicago sativa*): effects on lignin composition in specific cell types. *New Phytol* 179: 738–750; 2008 doi:10.1111/j.1469-8137.2008.02502.x.
- Novaes E.; Drost D. R.; Farmerie W. G.; Pappas G. J. Jr; Grattapaglia D.; Sederoff R. R.; Kirst M. High-throughput gene and SNP discovery in *Eucalyptus grandis*; an uncharacterized genome. *BMC Genomics* 309: 312; 2008.
- Novillo F.; Medina J.; Salinas J. *Arabidopsis* CBF1 and CBF3 have a different function than CBF2 in cold acclimation and define different gene classes in the CBF regulon. *Proc. Natl. Acad. Sci. U. S. A.* 104: 21002–21007; 2007 doi:10.1073/pnas.0705639105.
- O'Neill N. R.; Farr D. F. *Miscanthus* blight; a new foliar disease of ornamental grasses and sugarcane incited by *Leptosphaeria* sp. and its anamorphic state *Stagonospora* sp. *Plant Dis* 80: 980–987; 1996.
- Oraby H.; Venkatesh B.; Dale B.; Ahmad R.; Ransom C.; Oehmke J.; Sticklen M. Enhanced conversion of plant biomass into glucose using transgenic rice-produced endoglucanase for cellulosic ethanol. *Transgenic. Res* 16: 739–749; 2007 doi:10.1007/s11248-006-9064-9.
- Paredez A. R.; Persson S.; Ehrhardt D. W.; Somerville C. R. Genetic evidence that cellulose synthase activity influences microtubule cortical array organization. *Plant Physiol* 147: 1723–1734; 2008 doi:10.1104/pp.108.120196.
- Paredez A. R.; Somerville C. R.; Ehrhardt D. W. Visualization of cellulose synthase demonstrates functional association with microtubules. *Science* 312: 1491–1495; 2006 doi:10.1126/science.1126551.
- Paterson A. H.; Bowers J. E.; 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 A. K.; Chapman J.; Feltus F. A.; Gowik U.; Grigoriev I. V.; Lyons E.; Maher C. A.; Martis M.; Narechania A.; Ollilar R. P.; Penning B. W.; Salamov A. A.; Wang Y.; Zhang L.; Carpita N. C.; Freeling B.; Gingle A. R.; Hash C. T.; Keller B.; Klein P.; Kresovich S.; McCann M. C.; Ming R.; Peterson D. G.; Rahman M.; Ware D.; Westhoff P.; Mayer K. F. X.; Messing J.; Daniel S.; Rokhsar D. S. The *Sorghum bicolor* genome and the diversification of grasses. *Nature* 457: 551–556; 2009 doi:10.1038/nature07723.
- Paterson A. H.; Lin Y. R.; Li Z.; Schertz K. F.; Doebley J. F.; Pinson S. R.; Liu S. C.; Stansel J. W.; Irvine J. E. Convergent domestication of cereal crops by independent mutations at corresponding genetic-loci. *Science* 269: 1714–1718; 1995a doi:10.1126/science.269.5231.1714.
- Paterson A. H.; Saranga Y.; Menz M.; Jiang C. X.; Wright R. J. QTL analysis of genotype x environment interactions affecting cotton fiber quality. *Theor. Appl. Genet* 106: 384–396; 2003.
- Paterson A. H.; Schertz K. F.; Lin Y-R.; Liu S-C.; Chang Y-L. The weediness of wild plants: Molecular analysis of genes influencing dispersal and persistence of johnsongrass; *Sorghum halepense* (L.). *Pers. Proc. Natl. Acad. Sci. U. S. A.* 92: 6127–6131; 1995b doi:10.1073/pnas.92.13.6127.
- Patzlaff A.; McInnis S.; Courtenay A.; Surman C.; Newman L. J.; Smith C.; Bevan M. W.; Mansfield S.; Whetten R. W.; Sederoff R. R.; Campbell M. M. Characterization of a pine MYB that regulates lignification. *Plant J* 36: 743–754; 2003a doi:10.1046/j.1365-3113X.2003.01916.x.
- Patzlaff A.; Newman L. J.; Dubos C.; Whetten R. W.; Smith C.; McInnis S.; Bevan M. W.; Sederoff R. R.; Campbell M. M. Characterization of PtMYB1; an R2R3-MYB from pine xylem. *Plant Mol. Biol* 53: 597–608; 2003b doi:10.1023/B:PLAN.0000019066.07933.d6.
- Pavy N.; Pelgas B.; Beauseigle S.; Blais S.; Gagnon F.; Gosselin I.; Lamothe M.; Isabel N.; Bousquet J. Enhancing genetic mapping of complex genomes through the design of highly-multiplexed SNP arrays: application to the large and unsequenced genomes of white spruce and black spruce. *BMC Genomics* 9: 21; 2008 doi:10.1186/1471-2164-9-21.

- Pedersen J. F.; Vogel K. P.; Funnell D. L. Impact of reduced lignin on plant fitness. *Crop Sci.* 45: 812–819; 2005 doi:10.2135/cropsci2004.0155.
- Piedade M. T. F.; Junk W. J.; Long S. P. The productivity of the C4 grass *Echinochloa polystachya* on the Amazon floodplain. *Ecology* 72: 1456–1463; 1991 doi:10.2307/1941118.
- Pilate G.; Guiney E.; Holt K.; Petit-Conil M.; Lapierre C.; Lep le J-C.; Pollet B.; Mila I.; Webster E. A.; Marstorp H. G.; Hopkins D. W.; Jouanin L.; Boerjan W.; Schuch W.; Cornu D.; Halpin C. Field and pulping performances of transgenic trees with altered lignification. *Nature Biotechnol* 20: 607–612; 2002 doi:10.1038/nbt0602-607.
- Pimentel D.; Harvey C.; Resosudarmo P.; Sinclair K.; Kurz D.; McNair M.; Crist S.; Shpritz L.; Fitton L.; Saffouri R.; Blair R. Environmental and economic costs of soil erosion and conservation benefits. *Science* 267: 1117–1123; 1995 doi:10.1126/science.267.5201.1117.
- Qin F.; Sakuma Y.; Li J.; Liu Q.; Li Y. Q.; Shinozaki K.; Yamaguchi-Shinozaki K. Cloning and functional analysis of a novel DREB1/CBF transcription factor involved in cold-responsive gene expression in *Zea mays* L. *Plant Cell Physiol* 45: 1042–1052; 2004 doi:10.1093/pcp/pch118.
- Quinby J. R.; Karper R. The inheritance of three genes that influence time of floral initiation and maturity date in milo. *J. Am. Soc. Agron* 37: 916–936; 1945.
- Quinby J. R.; Karper R. E. Inheritance of height in sorghum. *Agron. J* 46: 211–216; 1954.
- Raghu S.; Anderson R. C.; Daehler C. C.; Davis A. S.; Wiedenmann R. N.; Simberloff D.; Mack R. N. Ecology. Adding biofuels to the invasive species fire? *Science* 313: 1742; 2006 doi:10.1126/science.1129313.
- Ralph J.; Guillaumie S.; Grabber J. H.; Lapierre C.; Barri re Y. Genetic and molecular basis of grass cell-wall biosynthesis and degradability. III. Towards a forage grass ideotype. *C. R. Biol* 327: 467–479; 2004 doi:10.1016/j.crvi.2004.03.004.
- Randall G. W.; Mulla D. Nitrate nitrogen in surface waters as influenced by climatic conditions and agricultural practices. *J. Environm. Qual* 30: 337–344; 2001.
- Ransom C.; Balan V.; Biswas G.; Dale B.; Crockett E.; Sticklen M. Heterologous *Acidothermus cellulolyticus* 1,4-beta-endoglucanase E1 produced within the corn biomass converts corn stover into glucose. *Appl. Biochem. Biotechnol* 137–140: 207–219; 2007 doi:10.1007/s12010-007-9053-3.
- Reddy G.; Upadhyaya H. D.; Gowda C. C. L. Current status of sorghum genetic resources at ICRISAT: their sharing and impacts. *J. SAT Agric. Res.* 2: 5; 2006 <http://www.icrisat.cgiar.org/Journal/archives.htm>.
- Reddy M. S.; Chen F.; Shadle G.; Jackson L.; Aljoe H.; Dixon R. A. Targeted down-regulation of cytochrome P450 enzymes for forage quality improvement in alfalfa (*Medicago sativa* L.). *Proc. Natl. Acad. Sci. U. S. A.* 102: 16573–16578; 2005 doi:10.1073/pnas.0505749102.
- Ribaut J. M.; Ragot M. Marker-assisted selection to improve drought adaptation in maize: the backcross approach; perspectives; limitations; and alternatives. *J. Exp. Bot* 58: 351–360; 2007 doi:10.1093/jxb/erl214.
- Ricaud C.; Land A.; Sullivan S. Losses from the recurrence of yellow spot epiphytotics in Mauritius. *Sugar Azucar* 75: 28–29; 1980.
- Rooney W. L.; Aydin S. Genetic control of a photoperiod-sensitive response in *Sorghum bicolor* (L.) Moench. *Crop Sci* 39: 397–400; 1999.
- Ruf S.; Hermann M.; Berger I. J.; Carrer H.; Bock R. Stable genetic transformation of tomato plastids and expression of a foreign protein in fruit. *Nat. Biotechnol* 19: 870–875; 2001 doi:10.1038/nbt0901-870.
- Saballos A. Development and utilization of sorghum as a bioenergy crop. In: Vermerris W. (ed) Genetic improvement of bioenergy crops. Springer, New York, pp 211–248; 2008.
- Salehi H.; Ransom C. B.; Oraby H. F.; Seddighi Z.; Sticklen M. B. Delay in flowering and increase in biomass of transgenic tobacco expressing the *Arabidopsis* floral repressor gene *FLOWERING LOCUS C*. *J. Plant Physiol* 162: 711–717; 2005 doi:10.1016/j.jplph.2004.12.002.
- Saranga Y.; Jiang C. X.; Wright R. J.; Yakir D.; Paterson A. H. Genetic dissection of cotton physiological responses to arid conditions and their inter-relationships with productivity. *Plant Cell Environ* 27: 263–277; 2004 doi:10.1111/j.1365-3040.2003.01134.x.
- Saranga Y.; Menz M.; Jiang C. X.; Wright R. J.; Yakir D.; Paterson A. H. Genomic dissection of genotype x environment interactions conferring adaptation of cotton to arid conditions. *Gen. Res* 11: 1988–1995; 2001 doi:doi:10.1101/gr.157201.
- Scheinost P. L.; Lammer D. L.; Cai X.; Murray T. D.; Jones S. S. Perennial wheat: a sustainable cropping system for the Pacific Northwest. *Am. J. Alternative Agric* 16: 147–151; 2001.
- Schmierer D. A.; Kandemir N.; Kudrna D. A.; Jones B. L.; Ullrich S. E.; Kleinhofs A. Molecular marker-assisted selection for enhanced yield in malting barley. *Mol. Breed* 14: 463–473; 2005 doi:10.1007/s11032-005-0903-9.
- Sharma P. N.; Torii A.; Takumi S.; Mori N.; Nakamura C. Marker-assisted pyramiding of brown panthopper (*Nilaparvata lugens* St l) resistance genes *Bph1* and *Bph2* on rice chromosome 12. *Hereditas* 140: 61–69; 2004 doi:10.1111/j.1601-5223.2004.01726.x.
- Smith D. R.; Quinlan A. R.; Peckham H. E.; Makowsky K.; Tao W.; Woolf B.; Shen L.; Donahue W. F.; Tusneem N.; Stromberg M. P.; Stewart D. A.; Zhang L.; Ranade S. S.; Warner J. B.; Lee C. C.; Coleman B. E.; Zhang Z.; McLaughlin S. F.; Malek J. A.; Sorenson J. M.; Blanchard A. P.; Chapman J.; Hillman D.; Chen F.; Rokhsar D. S.; McKernan K. J.; Jeffries T. W.; Marth G. T.; Richardson P. M. Rapid whole-genome mutational profiling using next-generation sequencing technologies. *Genome. Res* 18: 1638–1642; 2008 doi:10.1101/gr.077776.108.
- Somleva M. N.; Snell K. D.; Beaulieu J. J.; Peoples O. P.; Garrison B. R.; Patterson N. A. Production of polyhydroxybutyrate in switchgrass; a value-added co-product in an important lignocellulosic biomass crop. *Plant Biotechnol. J* 6: 663–678; 2008 doi:10.1111/j.1467-7652.2008.00350.x.
- Somleva M. N.; Tomaszewski Z.; Conger B. V. *Agrobacterium*-mediated genetic transformation of switchgrass. *Crop Sci* 42: 2080–2087; 2002.
- Steele K. A.; Price A. H.; Shashidhar H. E.; Witcombe J. R. Marker-assisted selection to introgress rice QTLs controlling root traits into an Indian upland rice variety. *Theor. Appl. Genet* 112: 208–221; 2006 doi:10.1007/s00122-005-0110-4.
- Sticklen M. Plant genetic engineering to improve biomass characteristics for biofuels. *Curr. Opin. Biotechnol* 17: 315–319; 2006 doi:10.1016/j.copbio.2006.05.003.
- Taylor L. E. II; Dai Z.; Decker S. R.; Brunecky R.; Adney W. S.; Ding S. Y.; Himmel M. E. Heterologous expression of glycosyl hydrolases in planta: a new departure for biofuels. *Trends Biotechnol* 26: 413–424; 2008 doi:10.1016/j.tibtech.2008.05.002.
- Thinggaard K. Study of the role of *Fusarium* in the field establishment problem of *Miscanthus*. *Acta Agric. Scand. B. Plant Soil Sci.* 47: 238–241; 1997.
- Vanholme R.; Morreel K.; Ralph J.; Boerjan W. Lignin engineering. *Curr. Opin. Plant Biol* 11: 278–285; 2008. doi:10.1016/j.pbi.2008.03.005.
- Vermerris W.; Saballos A.; Ejeta G.; Mosier N. S.; Ladisch M. R.; Carpita N. C. Molecular breeding to enhance ethanol production from corn and sorghum. *Stover Crop Sci* 47: 142–153; 2007.

- Vogel K. P.; Hopkins A. A.; Moore K. J.; Johnson K. D.; Carlson I. T. Winter survival in switchgrass populations bred for high-IVDMD. *Crop Sci* 42: 1857–1862; 2002.
- Wagoner P. Perennial grain development: past efforts and potential for the future. *Crit. Rev. Plant Sci* 9: 381–408; 1990 doi:10.1080/07352689009382298.
- Wang J.; Elliott J. E.; Williamson R. E. Features of the primary wall CESA complex in wild type and cellulose-deficient mutants of *Arabidopsis thaliana*. *J. Exp. Bot* 59: 2627–2637; 2008 doi:10.1093/jxb/em125.
- Welling A.; Palva E. T. Involvement of CBF transcription factors in winter hardiness in birch. *Plant Physiol* 147: 1199–1211; 2008 doi:10.1104/pp.108.117812.
- Wenzl P.; Carling J.; Kudrna D.; Jaccoud D.; Huttner E.; Kleinhofs A.; Kilian A. Diversity arrays technology (DART) for whole-genome profiling of barley. *Proc. Natl. Acad. Sci. U. S. A.* 101: 9915–9920; 2004 doi:10.1073/pnas.0401076101.
- Wong C. K.; Bernardo R. Genomewide selection in oil palm: Increasing selection gain per unit time and cost with small populations. *Theor. Appl. Genet* 116: 815–824; 2008 doi:10.1007/s00122-008-0715-5.
- Wurbs D.; Ruf S.; Bock R. Contained metabolic engineering in tomatoes by expression of carotenoid biosynthesis genes from the plastid genome. *Plant J* 49: 276–288; 2007 doi:10.1111/j.1365-3113X.2006.02960.x.
- Wyman C. E. What is (and is not) vital to advancing cellulosic ethanol. *Trends Biotechnol* 25: 153–157; 2007 doi:10.1016/j.tibtech.2007.02.009.
- Xu S. L.; Rahman A.; Baskin T. I.; Kieber J. J. Two leucine-rich repeat receptor kinases mediate signaling linking cell wall biosynthesis and ACC synthase in *Arabidopsis*. *Plant Cell* 20: 3065–3079; 2008 doi:10.1105/tpc.108.063354.
- Yang C.; Xu Z.; Song J.; Conner K.; Barrena G. V.; Wilson Z. A. *Arabidopsis* MYB26/MALE STERILE35 regulates secondary thickening in the endothecium and is essential for anther dehiscence. *Plant Cell* 19: 534–548; 2007 doi:10.1105/tpc.106.046391.
- Yilmaz A.; Nishiyama M. Y. Jr; Garcia-Fuentes B.; Souza G. M.; Janies D.; Gray J.; Grotewold E. GRASSIUS: a platform for comparative regulatory genomics across the grasses. *Plant Physiol* 149: 171–180; 2009 doi:10.1104/pp.108.128579.
- Yoshida M.; Liu Y.; Uchida S.; Kawarada K.; Ukagami Y.; Ichinose H.; Kaneko S.; Fukuda K. Effects of cellulose crystallinity, hemicellulose, and lignin on the enzymatic hydrolysis of *Miscanthus sinensis* to monosaccharides. *Biosci. Biotechnol. Biochem* 72: 805–810; 2008 doi:10.1271/bbb.70689.
- Zhong R.; Demura T.; Ye Z.-H. SND1, a NAC domain transcription factor, is a key regulator of secondary wall synthesis in fibers of *Arabidopsis*. *Plant Cell* 18: 3158–3170; 2006 doi:10.1105/tpc.106.047399.
- Zhong R.; Lee C.; Zhou J.; McCarthy R. L.; Ye Z. H. A battery of transcription factors involved in the regulation of secondary cell wall biosynthesis in *Arabidopsis*. *Plant Cell* 20: 2763–2782; 2008 doi:10.1105/tpc.108.061325.
- Zhong R.; Richardson E. A.; Ye Z. H. The MYB46 transcription factor is a direct target of SND1 and regulates secondary wall biosynthesis in *Arabidopsis*. *Plant Cell* 19: 2776–2792; 2007a doi:10.1105/tpc.107.053678.
- Zhong R.; Richardson E. A.; Ye Z. H. Two NAC domain transcription factors; SND1 and NST1; function redundantly in regulation of secondary wall synthesis in fibers of *Arabidopsis*. *Planta* 225: 1603–1611; 2007b doi:10.1007/s00425-007-0498-y.
- Zhong R.; Ye Z.-H. Regulation of cell wall biosynthesis. *Curr. Opin. Plant Biol* 10: 564–572; 2007 doi:10.1016/j.pbi.2007.09.001.
- Zhou R.; Zhu Z.; Kong X.; Huo N.; Tian Q.; Li P.; Jin C.; Dong Y.; Jia J. Development of wheat near-isogenic lines for powdery mildew resistance. *Theor. Appl. Gen* 110: 640–648; 2005 doi:10.1007/s00122-004-1889-0.
- Zhou Y. L.; Xu J. L.; Zhou S. C.; Yu J.; Xie X. W.; Xu M. R.; Sun Y.; Zhu L. H.; Fu B. Y.; Gao Y. M.; Li Z. K. Pyramiding *Xa23* and *Rxo1* for resistance to two bacterial diseases into an elite indica rice variety using molecular approaches. *Mol. Breed* 23: 279–287; 2009 doi:10.1007/s11032-008-9232-0.
- Zili Y.; Puhua Z.; Chengcai C.; Xiang L.; Wenzhong T.; Li W.; Shouyun C.; Zuoshun T. Establishment of genetic transformation system for *Miscanthus sacchariflorus* and obtaining of its transgenic plants. *High Tech. Lett* 10: 27–31; 2004.
- Zuo K. J.; Qin J.; Zhao J. Y.; Ling H.; Zhang L. D.; Cao Y. F.; Tang K. X. Overexpression GbERF2 transcription factor in tobacco enhances brown spots disease resistance by activating expression of downstream genes. *Gene* 391: 80–90; 2007 doi:10.1016/j.gene.2006.12.019.