



BIOMASS AND BIOFUELS SERIES

Bioenergy

Feedstocks

Breeding and Genetics

EDITED BY

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Preface

The world energy use grew by 39% from 1990 to 2008. It is estimated that the global demand for energy will increase by at least 50% over the next 20 years. Energy consumption growth of several developing nations remains vigorous. Hydrocarbons, petroleum, coal, and natural gas are now the chief sources of energy. All are finite resources and their natural reserves are depleting every day. In addition, during their conversion and use several greenhouse gases are emitted with a potential for climatic warming.

Bioenergy, both biofuels and biopower, produced from renewable sources are sustainable alternatives to hydrocarbons. Bioenergy use has the potential to lower greenhouse gas emissions, boost rural economy, and ensure energy security. Interest in bioenergy began in early 20th century but it was reinforced in the recent decades. Biopower includes co-firing bioenergy feedstocks with coal to reduce problem emissions. Due to government incentives during 1995–2005, commercial scale biofuels, mainly ethanol, became available in the European Union, UK, USA, Brazil, and many other countries around the world. Most of the biofuels are derived from corn grain, sugarcane, and vegetable oil feedstocks thus creating a food versus fuel controversy. Second-generation biofuels are now being made from nonfood, lignocellulosic materials such as municipal waste and wood chips, along with dedicated crops such as switchgrass and *Miscanthus*.

Plant breeding is critical for crop improvement. Due to intensive breeding efforts in both public and private sectors average maize grain yield has increased by 745% since 1930. Several of the dedicated feedstock crops, for example, switchgrass and *Miscanthus*, are only recently

removed from the wild and need serious breeding efforts for improvement. Increased biomass yield and improved quality through breeding efforts can make feedstock more economical and attractive. This book on *Bioenergy Feedstocks: Breeding and Genetics* should greatly contribute to these breeding efforts.

We are grateful to John Wiley & Sons, Inc. for their prudence and supporting us in publishing this book. Contribution from many prominent scientists on bioenergy research has greatly enhanced this publication. We extend our sincere appreciation to all the chapter contributors for their invaluable contribution. We also appreciate the efforts of all who directly or indirectly supported our endeavor. We sincerely believe that this book will be a useful reference for cultivar improvement of lignocellulosic biomass feedstock crops.

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Chapter 1

Introduction

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By most estimates, world population growth has more than tripled during the past 100 years, going from approximately 2–7 billion persons (Anonymous, 2012). To sustain the economies needed to support this type of unprecedented population growth, readily available, cheap, scalable, and efficient energy sources were required. These sources turned out to be hydrocarbons, both oil and coal, and after the Second World War, nuclear power. Heavy hydrocarbon use resulted in their depletion and increased cost and a concurrent increase in environmental problems due to gas emissions. Although “clean” as far as gas emissions, nuclear power has its own problems associated with safety and disposal of its highly toxic waste products. Therefore, alternative energy sources such as wind, solar, and bioenergy that are capable of offsetting some of the

hydrocarbons and nuclear use and mitigating their environmental problems are now being investigated and, in some cases, implemented on a commercial scale.

Lignocellulosic feedstocks derived from plant biomass emerged as a sustainable and renewable energy source that underpins the bioenergy industry (McLaughlin, 1992; Sanderson et al., 2006). Bioenergy, both biopower and biofuels, could contribute significantly to meet growing energy demand while mitigating the environmental problems. The Energy Independence and Security Act RFS2 in the United States mandates that annual biofuels' use increase to 36 billion gallons per year by 2022, of which 21 billion gallons should come from advanced biofuels (EISA, 2007). Waste products, both agricultural and forest residues, are obvious choices as base feedstocks; however, it is the use of “dedicated” energy crops where the ability to achieve the billion tons of biomass USA goals will be realized (Perlack et al., 2005). Several plant species such as switchgrass, Miscanthus, corn fodder, sorghum, energy canes, and other grass and legume species have demonstrated tremendous potential for use as dedicated bioenergy feedstocks especially for the production of advanced biofuels. Their adaptation patterns along most agro-ecological gradients also offer options for optimizing a crop species mix for any bioenergy feedstock production system.

1.1 Historical Development

The concept of bioenergy is not new. Early human civilization witnessed energy potential of plant biomass and used it in cooking and as a source of light. By 1912, Rudolf Diesel demonstrated that diesel obtained from plant biomass can be used in automobile operation (Korbitz, 1999). The shortage of crude oil during the 1970s reinforced

the world's motivation toward plant biomass as alternative energy source. In Brazil, use of ethanol to power automobile dates back to the late 1920s. Brazil's National Alcohol Program under government funding was launched in 1975 to promote ethanol production from sugarcane. In 2007, Brazil produced more than 16 billion liters of ethanol (Goldemberg, 2007).

In the United States, during the past decade, billions of dollars were invested annually by the federal and state governments, venture capitalists, and major private companies for the development of new technology to convert feedstock species into renewable biofuels. Major breakthroughs have happened during the past few years and the biofuel production increased significantly. Significant improvements have also noticed on conversion technologies thus moving the biofuel from pilot scale to near-commercial scale.

At present, biofuels are produced from corn grain, sugar cane, and vegetable oil. In the United States, corn is the main feedstock used to produce ethanol. In 2010, corn-based ethanol production was about 50 billion liters (USDOE, 2011). With the increasing world's food demand there is serious economic (animal feed costs are rising) and even ethical concern with using corn grain in ethanol production. In the mid-1985s, U.S. Department of Energy (DOE) Herbaceous Energy Crops Program (HECP), coordinated by Oakridge National Laboratory (ORNL), funded research to identify potential herbaceous species as potential bioenergy feedstock. Over 30 plant herbaceous crop species including grasses and legumes were studied, and consequently switchgrass was chosen as the "model bioenergy species" (McLaughlin and Kszos, 2005). Under optimum conditions, switchgrass demonstrated annual biomass yield as high as 24 Mg ha⁻¹, and each ton of biomass can produce about 380 L of ethanol (Schmer et al.,

2008). Carbon sequestration by 5-year-old switchgrass stand can add 2.4 Mg C ha⁻¹ year⁻¹ for 10,000 Mg ha⁻¹ of soil mass (Schmer et al., 2011). Other plant species with high bioenergy potential include Miscanthus, corn fodder, sorghum, sugarcane, prairie cordgrass, bluestems, eastern gamagrass, and alfalfa. Miscanthus hybrids have the potential to produce high biomass and can make a significant contribution to biofuel production and to the mitigation of climate change. Plant breeding will play an important role in improving the genetic potential of these species, as well as other potential species, and make them suitable as bioenergy feedstock.

1.2 Cultivar Development

Genetic improvement of plant species targeting biomass feedstock production, particularly the dedicated energy crops such as switchgrass and Miscanthus, is in a very early stage, posing both challenges and opportunities for genetic improvement. The current emphasis of most biomass feedstock cultivar development research is based on biomass yield. Due to extensive breeding efforts, maize grain yield has increased 745% from 1930 to the present (USDA-NASS, 2011). Biomass yield per unit of land is a function of many traits; thus plant breeders also have to address problems related to establishment, seed shattering, and resistance to abiotic/biotic stresses. Equally important is improvement in feedstock quality for sustainable bioeconomy. Research is still evolving on processes to convert biomass to bioenergy/biofuel that will dictate the quality targets of dedicated bioenergy crops. One likely scenario is that both enzymatic and thermochemical conversion technologies will be required depending on the biomass feedstock availability and the targeted bioenergy end product.

1.3 Breeding Approach

The fundamentals of feedstock cultivar development will be the same as ones that have been successfully employed in several agricultural crops for thousands of years. Most of the potential bioenergy crops are outcrossing polyploids and great genetic diversity exists both within and among populations. This reinforces the potential for genetic improvement of these crops. Most of the named switchgrass cultivars were developed only by seed increases of desirable plants identified from the wild or selected through two or three generations under cultivation (Casler et al., 2007). The improvement of quantitative traits will require several cycles of selection (Bouton, 2008). The traits that are qualitatively inherited can be improved rapidly. Exploitation of heterosis would require identification of genes involved in heterosis and development of heterotic pools, similar to the one that was followed in hybrid breeding in maize. Different crop species would need different plant breeding methodologies depending on their mode of reproduction, ploidy systems, and germplasm availability. For example, corn has a well-developed hybrid production system using inbred lines, which may not be directly applicable to crops like switchgrass that has nearly 100% self-incompatibility. Some species of *Miscanthus* and sugarcane that do not produce seeds require a different approach. The hundreds of years of experience gained in the development of modern cultivars of food and other agricultural crops can directly benefit the cultivar development research of bioenergy crops.

1.4 Molecular Tools

Rapid development in high-throughput genotyping, genotyping based on sequencing, and computational biology continues to shape modern plant breeding into a

new approach called “molecular breeding.” Rapid discoveries of DNA-based markers at significantly reduced cost have impacted cultivar development methodologies in the recent years. Advances in molecular biological research have uncovered several plant biological functions and enhanced the understanding of gene function at the molecular level (Bouton, 2008). Rapidly growing genome, transcriptome, proteome, and metabolome resources of several important biofuel crops can speed the process of feedstock development which can lead to improved economics of renewable bioenergy production. Lignin polymer is found to be interfering with enzymatic digestion of lignocellulosic biomass necessitating the pretreatment of biomass feedstock, making biofuel production an economic challenge (Dien et al., 2011). However, plant biologists have been able to characterize and modify lignin pathway and produce low lignin plants by silencing genes involved in lignin pathway (Dien et al., 2011; Fu et al., 2011). Transgenic technologies have also enabled plant breeders to look beyond target species for genes conferring desirable traits, but current regulatory aspects could curtail gains from transgenics, especially for bioenergy crops, without deregulation reforms that better balance both risk and benefit (Strauss et al., 2010).

1.5 Future Outlook

Changing climates as seen by frequent unprecedented drought cycles have become a serious challenge in the recent decades. This will require an “adjustment philosophy” in that breeding strategies will need to continually adjust trait targets for greater stress extremes with programs concentrating on stress tolerances growing in importance (Bouton, 2010). As biomass feedstock production scales up to a commercial level, there will also

be a significant shift in agricultural landscapes, leading to occurrence of new pest and diseases specific to the feedstock species. Exploration and exploitation of microbial endophytes implicated in protection of plants from a broad range of biotic and abiotic stresses are important areas for future research (Ghimire et al., 2011). Bioenergy crop breeders should therefore take proactive action to integrate all conventional and modern tools into their cultivar development research.

There are government policy issues that may assist the growth of bioenergy industry. However, these are political issues not within the scope of this book and will need to be hashed out at that level. But one thing is certain, bioenergy cultivar development research will benefit by always striving for a cost-effective product that competes in the free market with hydrocarbons and nuclear power. This should become more possible by leveraging facilities/resources established for traditional agricultural crops and implementation of regional/national/international collaborations between institutions involved in bioenergy feedstock research. Finally, sharing germplasms between participating institutes would help maintain genetic diversity of the breeding pools needed for long-term use.

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Chapter 2

Switchgrass Genetics and Breeding Challenges

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2.1 Introduction

Liquid biofuel production from biomass has the potential to reduce greenhouse gas emissions from transportation and dependence on fossil fuels extracted from politically volatile regions (Somerville, 2007; Bartley and Ronald, 2009; Vega-Sanchez and Ronald, 2010). Cultivated grasses are the most abundant sustainable class of biomass that can be produced in the United States (~57%, Perlack et al., 2005). Switchgrass (*Panicum virgatum* L.), in particular, is an attractive native species for development as a bioenergy crop given that largely unimproved varieties exhibit large biomass yield (up to 36.7 Mg ha⁻¹) and marked stress tolerance ([Figure 2.1](#); Thomason et al., 2004; McLaughlin and Adams Kszos, 2005; Bouton, 2007). Even with typical, lower-

yielding marginal land (5–11 Mg ha⁻¹), energy and emission measurements for switchgrass production give an approximately 5-fold net energy yield (output:input) and an approximately 10-fold reduction in greenhouse gas emissions compared with gasoline (Schmer et al., 2008). In order to realize greater benefits from the production of lignocellulosic fuels, there is an enormous need to apply various breeding methods and tools toward switchgrass improvement. Below, we outline switchgrass energy crop breeding goals and, in subsequent sections, provide an overview of the basic biology and genetic characteristics of switchgrass. We then discuss experiences and challenges related to switchgrass conventional and molecular breeding.

[FIGURE 2.1](#) A seed production field of the lowland switchgrass cultivar “Cimarron” in September, toward the end of the growing season, near Perkins, OK.



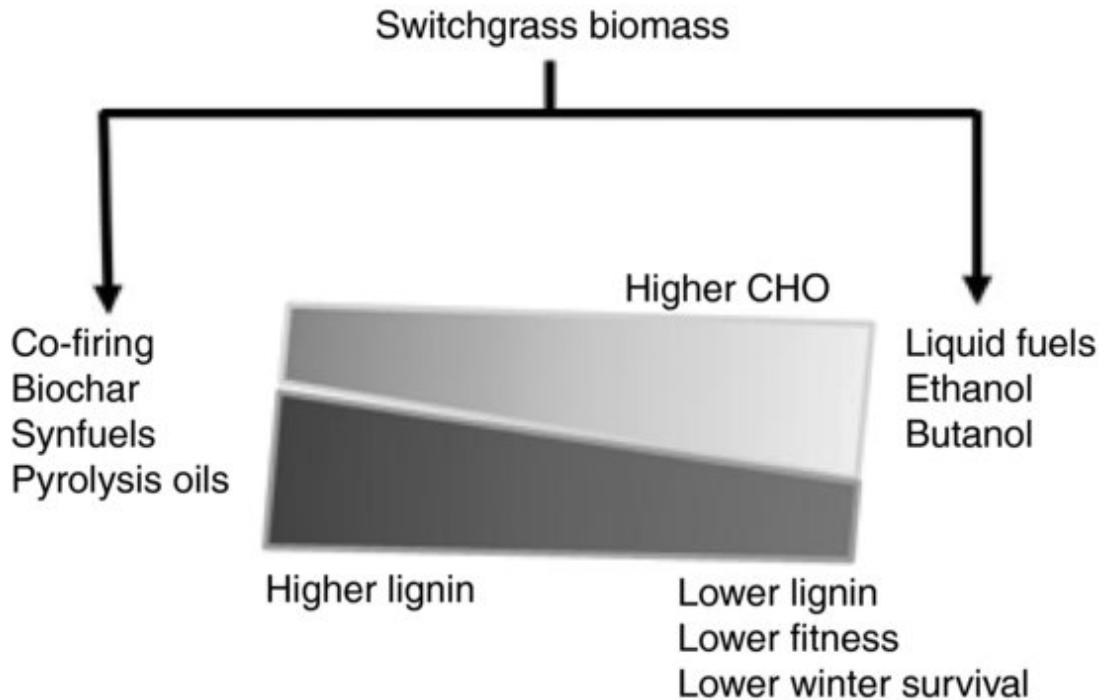
Biomass *yield* and *quality* are the two general classes of targets for genetic improvement of bioenergy crops. Selection of switchgrass for high biomass production is

ongoing (Vogel et al., 2010). Recently released cultivars “BoMaster,” “Cimarron,” and “Colony” produce higher biomass yields than the current best commercial cultivar “Alamo” and are primarily targeted for cellulosic feedstock production (Burns et al., 2008a, 2008b, 2010; Wu and Taliaferro, 2009). Similarly, high-performing replacement proprietary cultivars for old standards such as Alamo, “Kanlow,” and “Cave-in-Rock” are currently sold in commercial bioenergy seed trade as EG1101, EG1102, and EG2101, respectively (<http://www.bladeenergy.com/SwitchProducts.aspx>).

Abiotic and biotic stress tolerance and improved agronomic characteristics, such as reduced seed dormancy (Burson et al., 2009), are important for establishing and obtaining consistent biomass production. In terms of biomass quality, the goals for the two current biomass to biofuel conversion platforms, biochemical and thermochemical, are roughly opposite ([Figure 2.2](#)). For biochemical conversion methods that mostly produce alcohol fuels, the quality goal is to optimize the quantity of sugar that can be obtained from the biomass. For example, transgenic switchgrass mutants with reduced lignin content in the cell wall release a larger fraction of cellulose-derived glucose when subject to *in vitro* digestion (Fu et al., 2011a, 2011b; Saathoff et al., 2011a). On the other hand, thermochemical biomass conversion methods to yield syngas or bio-oil produce higher-quality products from lignin compounds compared with carbohydrates (Bridgwater and Peacocke, 2000). With the exception of high-lignin plants for thermochemical conversion, it is worth noting that many of the characteristics desirable for switchgrass production for biofuels are similar to those desirable for forage use (Sarath et al., 2008). However, as for forage-related traits, it is anticipated that a major challenge for switchgrass breeding for many biofuel quality traits will be the occurrence of trade-

offs between the genetic determinants for biomass quality and quantity.

FIGURE 2.2 Potential applications and consequences of changing switchgrass biomass quality. CHO, carbohydrate.



2.2 Origin and Distribution

Switchgrass is a polyploid, perennial C_4 warm-season grass native to the tallgrass prairies of the continental United States, with a historical distribution through Central America. Consistent with the hypothesis that switchgrass survived the Pleistocene Glaciations in southern refugia (McMillan, 1959), recent analyses suggest that the southeastern United States is the center of diversity for extant switchgrass populations (Zhang et al., 2011). Much of the original native stands have disappeared over time, mostly through agricultural activities; however, pockets of native stands do exist within the continental United States (Vogel et al., 2010). Panicums belong to the subfamily Panicoideae and can be considered to be monophyletic based on molecular phylogeny (Giussani