Trends in Plant Science

CellPress

Review

Molecular Breeding for Improved Second Generation Bioenergy Crops

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There is increasing urgency to develop and deploy sustainable sources of energy to reduce our global dependency on finite, high-carbon fossil fuels. Lignocellulosic feedstocks, used in power and liquid fuel generation, are valuable sources of non-food plant biomass. They are cultivated with minimal inputs on marginal or degraded lands to prevent competition with arable agriculture and offer significant potential for sustainable intensification (the improvement of yield without the necessity for additional inputs) through advanced molecular breeding. This article explores progress made in next generation sequencing, advanced genotyping, association genetics, and genetic modification in second generation bioenergy production. Using poplar as an exemplar where most progress has been made, a suite of target traits is also identified giving insight into possible routes for crop improvement and deployment in the immediate future.

Trends

Second generation bioenergy feedstocks cultivated from non-food crops on marginal lands are widely held as a promising source of renewable and sustainable energy to help displace fossil fuels.

A substantial yield gap exists for key bioenergy crops and this has held back their successful commercial deployment for reasons of economy and sustainability.

Affordable advanced molecular breeding techniques have driven progress in food crops in recent years as next generation sequencing has dramatically reduced costs.

Traits of interest for improvement in bioenergy include biomass yield, feedstock quality, drought tolerance, and pest resistance.

Genotyping by sequencing, association genetics, and genetic modification are now beginning to be applied in second generation bioenergy species with a view to addressing these breeding targets.

Molecular Breeding for Sustainable Intensification

In the past decade advanced breeding techniques have been increasingly employed to enhance commercially important traits in staple crops and livestock as food producers seek to improve the yield, economy, resilience, and environmental sustainability of their products [1]. Next generation sequencing (NGS), high-throughput genotyping, and **molecular breeding** (see Glossary) methodologies such as marker assisted selection (MAS), **genomic selection** (GS), and genetic modification (GM) have been applied in a significant number of important species. These include cereals such as rice (*Oryza sativa*) [2], maize (*Zea mays*) [3], and barley (*Hordeum vulgare*) [4]; other crops including potato (*Solanum tuberosum*) [5], apple (*Malus domestica*) [6], and soybean (*Glycine max*) [7]; and domestic livestock species including cattle [8] and pigs [9]. In total more than 100 plant genomes have been sequenced since 2000 [10] as costs for sequencing technology have plummeted and instrument capacity increased millionfold [11]. Targeted phenotypes vary depending upon the breeding priorities for a given species but tend to be broadly focussed on disease resistance [4]; maximising yields [7]; improving nutritional quality [3]; reducing waste [9], or inducing tolerance to more challenging environmental conditions such as drought and salt stress [12]. Many of these breeding approaches may enable us to realise the 'more from less' paradigm suggested as part of the sustainable intensification of crop production. Here we take the Royal Society's definition of sustainable intensification as a system, 'in which yields are increased without adverse environmental impact and without the cultivation of more land' and suggest that primary productivity should be refocused towards these goals [13]. There remains considerable potential to better exploit genetic resources where the development of new and novel crops is focussed away from yield *per se* and towards improved quality of product with reduced inputs and waste. For **bioenergy** crops, that have hardly been selected and bred in the past, this provides a framework to rapidly harness the power of new DNA

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technologies to deliver higher, more sustainable yields that are of wide value to society. Such crops include perennials trees including poplar (*Populus*) and willow (*Salix*) and grasses such as *Miscanthus* (*Miscanthus sinensis*, *Miscanthus sacchariflorus*, *Miscanthus* × *giganteus*), *Arundo donax*, and switchgrass (*Panicum virgatum*); grown for their **lignocellulosic** biomass.

The progress of bioenergy has been hampered by controversy surrounding first generation biofuels derived from food crops, with public opposition over their suggested impact on food security and debate as to the true extent of the benefit they provide over conventional fossil fuels. Second generation lignocellulosics are considered as promising candidates for the production of sustainable, cost-effective bioenergy feedstocks, but in contrast to food crops, a relatively short time and limited effort has been focussed on their breeding and improvement with very little commercial deployment to date despite the clear advantages they confer over the first generation. The key question is whether the opportunity provided by new DNA technologies can help enable the accelerated development of better lignocellulosic crops. Such crops require research effort to maximise their inherent potential for competitive yields, reduced greenhouse gas (GHG) emissions, and favourable energy balances relative to the first generation bioenergy and fossil fuels it is hoped they will replace [14]. Thus the central aim for bioenergy breeding must be sustainable yield intensification, that is, increasing biomass production per land unit area without environmental degradation or increased agronomic inputs.

The Yield Gap in Second Generation Bioenergy

The yield gap may be considered as the difference between potential and actual yield, where potential yield (Yp) is defined as the yield obtained if the full genetic potential of a crop is realised under optimal conditions in which water and nutrients are non-limiting and biotic stresses are controlled (often only seen in experimental conditions). Actual yield (Ya), by contrast, is the average yield obtained in the field and the yield gap is calculated as the difference between Yp and Ya [15]. Yield gap analyses are commonly employed for food crops including rice [16], maize [17], and cassava (*Manihot esculenta*) [18] and consider the impact of pests and disease, water availability, and soil nutrients on crop performance in a given climate or location. As such, they are considered important for the sustainable intensification of agriculture by highlighting which crops and regions have the greatest potential for increased productivity and identifying research priorities for crop improvement [15].

Figure 1 illustrates the range of biomass yields obtained in published field trials for poplar, willow, and *Miscanthus*. Unsurprisingly the highest yields are generally achieved in trials with supplemental irrigation and/or fertiliser application and the breadth of reported yields is suggestive of a significant yield gap for these key lignocellulosic bioenergy crops. While potential yields are rarely achieved, the possibility exists for targeted molecular approaches to help overcome this gap in a timely manner by addressing traits that can drive the sustainable intensification of second generation biomass. These data suggest a yield gap of approximately 15 tonnes ha^{-1} year^{-1}, providing a significant gap that may be addressed using molecular breeding for the accelerated deployment of these relatively new crops.

Key Traits for the Sustainable Intensification of Bioenergy

To pursue the sustainable intensification of biomass, research must target traits that can deliver increased yields while minimising the need for agronomic inputs, that is, supplementary irrigation, fertilisation, and pesticide application. Performance must also be consistent, with perennial bioenergy crops able to provide reliably high yields over multiple harvest cycles in the face of variable and changing climatic conditions. Although grain yield and not biomass yield is key to food crop improvement, many of the traits that represent breeding priorities for bioenergy are also relevant to food (pest and disease resistance, drought and salinity tolerance, and nitrogen and water use efficiencies). These traits underpin the delivery of sustainable intensification for

Glossary

Bioenergy: renewable energy derived from biomass, generally plant material or organic waste. Examples of bioenergy include liquid biofuels for transport, biomass for combustion or biogas.

Genotyping-by-sequencing (GBS): the resequencing of multiple genomes of a species to discover large numbers of SNPs for GWAS or genomic selection.

Genomic selection (GS): requires phenotyping in a large training population followed by assignment of breeding values for individuals based solely on genotyped, trait-associated markers. Unlike in GWAS the effects of all genetic markers are estimated simultaneously.

Genome editing: the use of engineered nucleases to execute precise insertions, alterations, or deletions from the target genome. The CRISPR/Cas system is a powerful new tool for genome editing in eukaryotes.

Genome-wide association study (GWAS): a forward genetic approach; GWAS uses DNA markers across the genome of a species and seeks statistical associations between markers and traits of interest, which can inform candidate gene identification and marker assisted breeding.

Lignocellulosics: second generation bioenergy feedstocks derived from plants and comprising the glucose polymer cellulose and associated phenolic polymer lignin that constitute the majority of the secondary cell wall in such species.

Molecular breeding: the application of molecular biology tools especially DNA markers to plant or animal breeding. These include QTL mapping, gene discovery, marker assisted selection (MAS), genomic selection (GS), and genetic modification (GM).

Single nucleotide polymorphism (SNP): a DNA sequence variation caused when a single nucleotide (A, T, C, or G) varies at a given position in the genome between members of the same species.