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Molecular breeding of switchgrass for use as a biofuel crop

Joseph H Bouton

Switchgrass (*Panicum virgatum* L.) is projected to become one of the main herbaceous, biofuel crops in United States. This status was the result of several years of research; much it sponsored by the United States Department of Energy (DOE). Literature documenting fundamental aspects of switchgrass taxonomy, genetics, breeding, management, physiology, and use is now available and form the basis for protocols to establish and manage the crop, as well as efforts to develop improved cultivars. Future improvement will include production of high yielding hybrids and the use of genomic and transgenic biotechnologies to enhance both productivity and chemical composition. Reducing bioconversion recalcitrance via reduction of lignin content is an example of projected future research in this area.

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Introduction

Switchgrass is a component of the North American grasslands, that along with indiangrass [*Sorghastrum nutans* (L.) Nash], and big bluestem (*Andropogon gerardii* Vitman), compose the 'big three' grasses because of their being the predominant species found in tall grass prairies. Its current uses include sowing to restore native range, to serve as a component of conservation reserve program (CRP) lands, and for use in highly managed pasture or hay production systems. However, its workmanlike status is changing substantially as it becomes one of the main herbaceous bioenergy crops for production of cellulosic biofuels.

This role of the main herbaceous biofuel crop was not reached lightly, but was the result of several years of DOE sponsored research projects that have now been summarized in an excellent paper by McLaughlin and Kszos [1••]. This paper chronicles the DOE's Bioenergy Feedstock Development Program (BFDP) at its Oak Ridge National Laboratory. One of the main goals of the BFDP was to select the most promising plant species for use as bioenergy feedstocks. As a result of this process, switchgrass was chosen as the main herbaceous because of its high yield, perennial nature, its soil and wildlife enhancing ability, ability to be established from seed, its status as a native grass, and its adaptability to poor soils and marginal cropland. The BFDP sponsored switchgrass research projects also resulted in a reduction of the crop's projected production costs by 25%, developed improved cultivars with higher yield, and recorded the crop's high carbon sequestration potential. Unfortunately, the DOE de-emphasized its crop production research in 2002, and BFDP was basically eliminated.

However, everything changed again with the USDA and DOE survey [2] documenting the need for herbaceous biofuel crops like switchgrass in order to reach the goal of replacing USA petroleum supplies with biofuels. This survey re-opened the door for increased investment into the genetic improvement of switchgrass as a biofuel feedstock. This current review will focus on the most recent series of papers and articles that have been published since the elimination of the BFDP.

Characteristics, management, production, and use

Vogel [3••] documented all aspects of switchgrass taxonomy, genetics, breeding, management, physiology, growth, and use. Switchgrass is an erect, C4 perennial grass that can reach up to 4.0 m in height (Figure 1a). Perennials like switchgrass have advantages over annual crops in saving of resources needed for re-planting and by protecting land from erosion by not exposing the soil to the rigors of plowing. Switchgrass also grows as a 'clonal modular organism' [4]. The tiller forms the main clonal growing module while the subunit of the tiller is the phytomer or 'shoot' which consists of a leaf blade and sheath, axillary bud, stem node and internode, and ligule (Figure 1b). Most switchgrass genotypes possess short underground stems called rhizomes that allow it to form a loose sod over time. Tillers can be vegetative or reproductive when the shoot bears a seedhead (Figure 1c and d). It has a diffuse panicle type seedhead with its spikelets positioned at the end of long branches. Spikelets, or the basic subunit of the seedhead, are two flowered (Figure 1e and f). The second floret is fertile and is where the seed is produced while the second is staminate. The lemma and palea are indurate and adhere tightly to the true seed or caryopsis at harvest (Figure 1g).

Biomass differences among cultivars are mainly because of number of phytomers per tiller and weight per phytomer

Figure 1

(a) Young (10-week-old) switchgrass plant growing in the field; **(b)** groups of tillers at base of switchgrass plants showing individual photomers; short rhizome indicated with arrow; **(c)** vegetative stems of switchgrass; **(d)** seedhead (panicle) of switchgrass; **(e)** spikelet of switchgrass showing two florets (flowers) with lower one staminate (male only) and upper one perfect (possessing both male and female parts); **(f)** perfect floret showing ovary (female part) and anthers (male part) and the enclosing lemma and palea bracts (each mark on the graduated scale is 1 mm); **(g)** mature switchgrass seed from left to right: mature seed at time of harvest, harvested seed with lemma and palea still adhering to the caryopsis or mature fertilized ovary now containing seedling embryo, and the caryopsis removed from the adhering lemma and palea (each mark on the graduated scale is 1 mm).

[4]. The frequency of reproductive tiller production, number of phytomers per tiller, and rate of phytomer development were also found to be important for switchgrass biomass and seed production [5]. Lignocellulosic yield, or the yield of only the lignin, cellulose, and hemicellulose components, is reported to parallel dry matter yield, which is total yield of all harvested components with only the water removed, so improvements in dry matter yield should be part of all future breeding efforts [6]. When evaluated for bioconversion potential, or the ability of the biomass to be converted to ethanol, switchgrass was found to be higher than alfalfa (*Medicago sativa* L.) in total carbohydrates on a weight basis, but its recovery was inversely correlated to maturity and lignin content [7].

Cropping with glyphosate tolerant (a.k.a. Roundup Ready®) soybeans (*Glycine max*) the year before spring planting was recently found to be a good approach to establishing the crop for highly managed conditions [8]. However, since switchgrass is a predominate component of CRP lands, strategies to convert these lands to productive stands for biofuel production is also being pursued. In this regard, harvesting these CRP stands once per year after a killing frost, and applying, from a normal grass hay production standpoint, low nitrogen fertilizer rates of 56 kg N ha^{-1} (e.g. application of 56 kg of actual nitrogen per hectare of land) was reported to be an effective management system for lands enrolled in, or managed like, those the CRP program [9].

Switchgrass production is not particularly limited by biotic pests. This situation will surely change as it is produced over large acreages in monoculture. Herbage feeders such as grasshoppers (Acididae family) and diseases such as panicum mosaic virus, spot blotch (*Helminthosporium sativa* Pam.), and rust diseases (causal agents *Uromyces graminicola* Burn. and *Puccinia graminis*) are already reported to be a problem for growing the species [3••]. More recently, *Bipolaris oryzae*, which normally causes brown spot and seedling blight in rice, was found to be a potential new disease of switchgrass [10]. However, at this stage, insecticides and fungicides are currently not used to any extent when establishing and managing switchgrass.

Switchgrass will be processed mainly as a cellulosic feedstock for different bioconversion processes including ethanol production or co-firing with coal [1••]. Although current USA ethanol is produced mainly from corn grain, producing ethanol from cellulosic materials will be important as the industry progresses because of the fact that cellulosic conversion has a higher ethanol yield advantage per unit area and exhibits a net energy content three times that of corn grain while emitting low levels of greenhouse gases [11•]. Cellulosic feedstocks are comprised of lignin, hemicellulose, and cellulose and are collectively called lignocellulosic materials. There are three main bioprocesses for converting lignocellulic feedstocks to ethanol; acid hydrolysis, enzymatic hydrolysis, and thermochemical [12•]. Acid hydrolysis is the most common process to release sugars necessary for fermentation with the main acid step being either dilute acid with high pressure or concentrated acid with low pressure. Although there may be a mild acid pre-treatment step in the enzymatic process, microbes containing cellulase enzymes are used to produce the primary fermentable sugars. For thermochemical, the feedstock is first gasified into a synthesis gas (e.g. mixture of hydrogen and carbon) that is then bubbled through fermenters containing microbes capable of converting the synthesis gas to ethanol. Finally, the synthesis gas has an additional potential to be catalyzed into other alcohols such as butanol that can similarly be used as a biofuel. This catalytic process is called the Fischer–Tropsch synthesis (DOE URL: http://www1.eere.energy.gov/biomass/catalytic_conversion.html).

For all bioconversion processes, the main objective for switchgrass production in the short run is dry matter yield, but ease of conversion to ethanol and total alcohol yield will be important in the long run for fermentation based bioconversion. However, at this time, no one-bioconversion process has emerged as a clear bioprocessing leader. Some of the first demonstration scale plants to be funded through the DOE are based on fermentation technologies, although thermo-chemical processes are also being used (USDA, DOE News Release; URL: http://www.energy.gov/news/4827.htm).

With all the discussion surrounding global climate change, sequestering CO_2 as carbon in underground root and rhizome biomass, also called 'carbon storage sinks', will be important. Results indicate switchgrass plantings have great potential to store significant amounts of soil carbon [13•].

Breeding and crop improvement

Although biomass yields for switchgrass vary depending on cultivar, year, and location, there is also a great potential to improve yield, as well as other traits that should add value to its use as a biofuel feedstock, through breeding and biotechnology research.

One very important aspect of switchgrass taxonomy and reproductive behavior is its grouping into two distinct ecotype categories: lowland and uplands [3••]. Lowlands are tall, coarse plants with exceptional biomass yields, and generally found in wet areas with mild winter temperatures. Lowlands are predominately tetraploids ($2n = 4\times = 36$). Uplands are shorter in stature than lowlands, lower in biomass, mainly collected in drier, colder zones, and are mainly octoploids ($2n = 8\times = 72$) with some tetraploids. No natural crossing occurs across the ploidy groups therefore upland and lowland types are reproductively isolated for the most part.

Further subdividing these groupings (subecotypes) into northern uplands, southern uplands, northern lowlands, and southern lowlands is also reported based on latitudinal adaptation [14]. Previously, chloroplast DNA was used to classify two distinct cytotypes, L or U, that similarly matched the lowland (L) or upland (U) classifications [3••]. Recently, analysis of the sequence alignments of the chloroplast intron *trnL*(UAA) in 34 switchgrass accessions revealed a deletion of 49 nucleotides (Δ350–399) in this intron that also appeared to be specific to lowland accessions [15]. These distinct cytotypes should be useful as a DNA marker for the classification of upland and lowland switchgrass germplasm, especially since DNA can be extracted directly from the seed without having to spend time and resources on growing plants, and also to determine hybrids between upland and lowland germplasm.

Genetic diversity is fairly high in switchgrass with the main collections accessible through the GRIN system (USDA-ARS Germplasm Information Network; URL: http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?26657). However, there is a relatively short list of current switchgrass cultivars with the main ones being Alamo, Blackwell, Cave-in-Rock, Dacotah, Forestburg, Kanlow, Nebraska 28, Shawnee, Summer, Sunburst, Pathfinder, and Trailblazer [16]. One can then draw the obvious conclusion that the species is barely removed from the wild from a crop improvement standpoint especially when compared to corn (*Zea mays* L.) and other high value forage crops such as alfalfa.

Initial cultivar development centered on accession or ecotype collections, screening these in field trials for performance and geographic adaptation, and then directly increasing seed and releasing the best accession population as a new cultivar [3••]. Cultivars such as Blackwell and Nebraska 28 were released in this manner. However, since switchgrass is cross-pollinated perennial, recent breeding methodologies include population improvement with the eventual development of synthetic cultivars, and the possible production of F1 hybrid cultivars [3••,17•].

Significant phenotypic variation exists within and between existing cultivars and ecotype collections for yield and forage quality parameters such as cell wall digestibility which is defined as the ability of rumen microbes to convert the cellulose and hemicellose of the fiber fraction into more simple sugars and starches useful for energy by the animal [3••,18–21]. Heritability for biomass yield and digestibility was estimated to be high enough to allow most researchers to predict and/or demonstrate adequate gain from selection especially when selection was based on half-sib family performance which is defined as the performance of progeny from different plants that have a single pollen parent in common [3••,22,23].

The effectiveness of using the honeycomb planting design to identify superior switchgrass genotypes in a population improvement program with a goal of improving biomass production was evaluated [24]. The main aspect in honeycomb selection is the removal of competition between genotypes. Inter-genotypic competition is usually eliminated by increasing the spacing between plants. The results of this study suggested that it is possible to make reasonable progress in identifying high biomass yielding switchgrass genotypes, both among and within families, at a plant spacing of 1.2 m using the honeycomb selection method [24]. Initial results of experimental cultivars developed using this selection approach demonstrated up to a 33% yield enhancement over Alamo and Kanlow parents when tested over years and locations in Georgia [25].

Are hybrids possible?

Hybrid cultivars capitalize on heterosis or the superiority of the hybrid individuals compared to their parents. This approach has been successfully employed by the corn seed industry with nearly all corn production resulting from planting these high yielding and uniform hybrids. Use of F1 hybrids in switchgrass would have the potential of dramatically increasing the biomass yield of switchgrass if a successful system of commercial production could be employed [17•].

Switchgrass is highly self-incompatible employing two systems to insure that cross pollinated seed is produced; one happens during pre-fertilization with a system similar to the S–Z gametophytic incompatibility system, and the other is a post-fertilization system that prevents intermating across ploidy levels [26]. The two independent, polyallelic genes, S and Z, when found in the pollen of most grasses must complementary and be compatible with the same genes in the style of the ovary. If not, pollination will not be successful. Hexaploids are very rare in nature, while controlled crosses between octoploids and tetraploids results in only a very low frequency of hexaploid progeny, leading to the conclusion that interploidy crosses are prevented in switchgrass probably because of an endosperm imbalance. High heterosis is also found when single cross hybrids were made from specific, clonally replicated genotypes [17•]. Clonal replication using tissue culture is also possible for large-scale production of the individual genotypes, which should allow their use in a commercially viable seed production program [1••]. These three characteristics demonstrate the potential for developing high yielding single cross hybrids of switchgrass.

To scale up for commercial seed production using this approach, thousands of switchgrass plantlets are produced through tissue culture of the lower stem nodes from highly self-incompatible parent plants with previous history of high heterosis [17•]. These clones are brought to field-ready status within a short period of time. Two superior parent plants propagated in this manner are then placed into isolated crossing blocks with the resultant seed being exclusively F1 hybrids.

Biotech approaches to switchgrass improvement

Cultivar development in switchgrass now includes the new and evolving areas of genomic and transgenic technologies. However, only limited reports are available.

Studies on genetic variation via molecular markers reported that upland and lowland ecotypes fell into their distinctive ecotype classes regardless of ploidy level [15,27]. Extensive genetic variation and polymorphism between upland and lowland switchgrass types, as well as within each type, were also observed. Finally, a relatively high genetic identity within populations, and limited evidence of recent hybridizations between the lowland and upland populations, were found.

Genomic tools for switchgrass are also limited and need to be developed. There are currently no reports of trait mapping in switchgrass. EST and genomic microsatellites are being developed for switchgrass [28,29] and should be a good source of molecular markers. The only publicly available switchgrass genetic map is the tetraploid ($2n = 4\times = 36$) cross between a genotype from the lowland cultivar Alamo and one from the upland cultivar Summer [30•]. It possesses only 102 RFLP markers distributed over eight homoecology groups, but does provide for a limited framework map for future mapping. It was also inferred from these results that segregation distortion is very common in switchgrass and the genomic constitution of this species is likely to be an autotetraploid with high degree of preferential pairing between homologous chromosomes. For the future, it will be necessary that useable genetic maps of switchgrass be constructed and made available for mapping value added traits useful to breeding programs. Establishing the relationship of the switchgrass genome with that of rice (Oryza sativa L.), where genomic sequences are available, is also a good approach.

The mapping population mentioned above [30•] was recently expanded in size and is currently maintained at the Samuel Roberts Noble Foundation. Microsatellite markers developed from conserved grasses, tall fescue, and switchgrass ESTs were assessed on parents and a subset of this mapping population and were found amplify sufficiently for use in future switchgrass mapping (M. Saha *et al.*, abstract 1A-12, 29th Symposium on Biotechnology for Fuels and Chemicals, Denver, CO, 29 April–2 May 2007; URL: http://www.simhq.org/meetings/29symp/29fuelsdraft_WEB.pdf).

Foreign genes were successfully transferred into switchgrass via the *Agrobacterium*-mediated transformation method [31]. These findings opened the door for using transformation to incorporate value-added genes that cannot be transferred through crossing and selection. Incorporating transgenes into a cross-pollinated, native plant like switchgrass will be problematic from the standpoint of possible transgene flow into feral populations. Therefore, any transgene will need to be one that has high impact to either the grower or the bioprocessing plant, but does not cause environmental harm. One such trait would be reduced lignin content because it removes the negative impact on the cell wall digestion, but plants with reduced lignin should be less environmentally fit because of their increased pest problems.

A transformation project funded by USDA and DOE with a goal of downregulation of lignin pathway genes in switchgrass is currently underway (Noble Foundation Press Release; URL: http://www.noble.org/Press_Release/ForageImprovement/BiomassGrant/index.html). The potential impact of this approach is seen with the comparison of different alfalfa transgenic plants downregulated for lignin pathway genes [32•]. Some of these transgenic alfalfa plants produced nearly twice as much sugar from cell walls as wild-type plants leading these researchers to conclude that lignin modification could even bypass the need for acid pretreatment and thereby facilitate bioprocess consolidation. They also speculated that bioenergy crops such as switchgrass are potential candidates for this approach.

Conclusions

Switchgrass has had an interesting history of moving from native range plant to that of one of the main herbaceous dedicated energy crops in the USA. This status was the result of a great deal of basic and applied research; much of it sponsored by the DOE. Because of these efforts, literature is available summarizing all aspects of switchgrass taxonomy, genetics, breeding, management, physiology, and use. This literature now forms the basis for current efforts to sow and establish the crop, as well as breeding programs whose goal is development of improved cultivars; even F1 hybrids are now a possibility.

For switchgrass to rise to the importance of crops like corn, however, more research will need to be conducted that incorporate biotechnologies into improvement programs. Recent grant announcements from the DOE-USDA biomass genomics research program includes projects with switchgrass as the main species (USDA, DOE News Release; URL: http://genomicsgtl.energy.gov/research/DOEUSDA/), that along with the establishment of a new bioenergy center concentrating on improving switchgrass recalcitrance as one of its major goals (DOE Bioenergy Research Center Fact Sheets; URL: http://www.science.doe.gov/News_Information/News_Room/2007/Bioenergy_Research_Centers/DOE%20BRC%20fact%20sheet%20final%206-26-07.pdf), are noteworthy. The future is therefore bright for switchgrass as a dedicated energy crop with millions of hectares projected to be planted in order to meet DOE goals [33].

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