Chapter 2
Switchgrass Breeding, Genetics, and Genomics

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Abstract Switchgrass was one of the dominant species of the North American tallgrass prairie and savanna ecosystems that once dominated a large portion of the continent. It is currently used for pasture, hay production, soil conservation, and biomass production for conversion to energy. Switchgrass was selected in 1992 as the herbaceous model species to develop dedicated cellulosic bioenergy crops. Breeding and genetics studies began on switchgrass in the 1950s, focused on utilization in livestock agriculture. Recent developments have rapidly increased the rate of gain for biomass yield, largely by increasing the focus and intensity of selection and improving the choice of germplasm and selection methods. Modern genomics tools are rapidly being incorporated into switchgrass breeding programs to increase the rate of gain for important agronomic and bioenergy traits, as well as to create new variability that can be captured in commercial cultivars.

2.1 Introduction

Switchgrass is a highly versatile grass, used for soil and water conservation, livestock production, and biomass production for conversion to energy. The species is native to North America, east of the 100th meridian, ranging from southern Canada to northern Mexico. It was once one of the dominant species of the tallgrass prairie and associated ecosystems that included savanna, sand barrens, forest margins, and grassland–wetland transition zones. The most significant taxonomic division within switchgrass occurs at the ecotype level and is related to

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habitat. Upland and lowland ecotypes were named largely for an obvious phenotypic differentiation that was originally associated with habitat. Upland ecotypes were found on upland sites that were subject to occasional or frequent droughts, while lowland ecotypes were found on lowland sites that were prone to seasonally wet soils. The upland–lowland taxonomic division figures prominently in nearly all the cultivation and breeding history of this species.

2.2 Biogeography

Switchgrass germplasm has been preserved in remnant prairies and associated ecosystems throughout its historic range (Fig. 2.1). While less than 1% of the tallgrass prairie and associated savanna ecosystems have been preserved, the species native to these habitats have been preserved in thousands of remnant sites [1]. Most of these sites have never been developed or plowed, but some represent abandoned farmlands, which have been allowed to return to their native habitat and species assemblage. Presumably the native species on these sites have been restored to these sites as a result of viable seed banks. In some cases, human intervention has been used to assist recovery, in the form of seeds introduced from other sites.

Prairie and savanna remnants in North America have been preserved under the leadership and custodianship of a wide range of organizations, including the U.S. Forest Service, many state agencies responsible for preservation and use of natural resources (e.g. State of Wisconsin Department of Natural Resources), non-governmental agencies such as The Nature Conservancy, and numerous private organizations that include railroad right-of-ways, rural cemeteries, and private conservationists. The size of these remnants ranges from tiny family cemeteries of several hundred square meters up to several national grasslands, some of which exceed 0.5 M ha in size (e.g. Cimarron, Comanche, Rita Blanca, and Black Kettle National Grasslands). These grasslands are highly variable in species composition, due to variations in climate and soil type, with switchgrass occupying a range of positions from the dominant species to rare or absent in many cases.

Switchgrass is a highly polymorphic species with considerable morphological and physiological variation that is closely related to climatic factors. It is highly photoperiodic along its north–south adaptation range. In their native habitat, northern accessions may flower as early as late June or early July after only 3–4 phytomers have been produced. Conversely, accessions from the extreme southern portion of the range may flower as late as mid-October after production of 7–10 phytomers. Photoperiodism and extreme flowering times have created a strong adaptation gradient associated with both photoperiod and temperature. Most recommendations are to move switchgrass germplasm no more than one hardiness zone (5°C increments) north or south of its origin to avoid stand loss. Northern germplasm is insufficiently heat tolerant and too early in flowering to be productive at southern locations, while southern germplasm may lack sufficient cold tolerance to survive at northern locations [2–4].

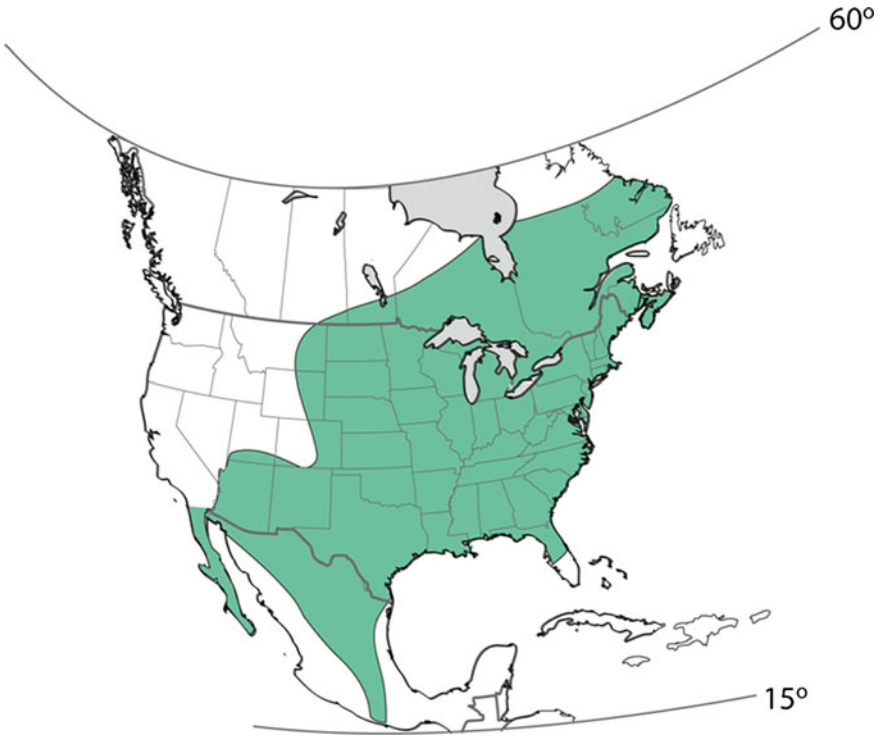

Fig. 2.1 Historical range of switchgrass in North America ([92], reprinted with permission)

Two ecotypes form the principal taxonomic division within switchgrass (Fig. 2.2). These two ecotypes were originally described based on observations of a distinct polymorphism associated with upland and lowland habitats, hence their names: upland and lowland ecotypes. Upland ecotypes are widely adapted north of 34°N latitude, extending into much of eastern Canada, but extremely rare at latitudes below 34°N. Lowland ecotypes are widely adapted up to approximately 42°N in the western portion of the range, but can be found as far north as 45°N in eastern North America due to climate-moderating oceanic effects. Upland and lowland ecotypes have generally been differentiated on the basis of plant phenotype: lowland plants are taller, have fewer and larger tillers, longer and wider leaves, thicker stems, and are later in flowering than upland plants (Table 2.1). Most lowland ecotypes also have a distinct blue coloring on stems and leaves, believed to be due to a waxy bloom on the epidermis. The blue hue is easily removed from some genotypes by touching or rubbing plant tissue between two fingers. As suggested by their names, upland ecotypes tend to be more drought tolerant than lowland ecotypes [5].

Upland and lowland switchgrass diverged on the evolutionary tree of life approximately 0.8–1.0 Mya [6–8]. Since that time, there have been approximately

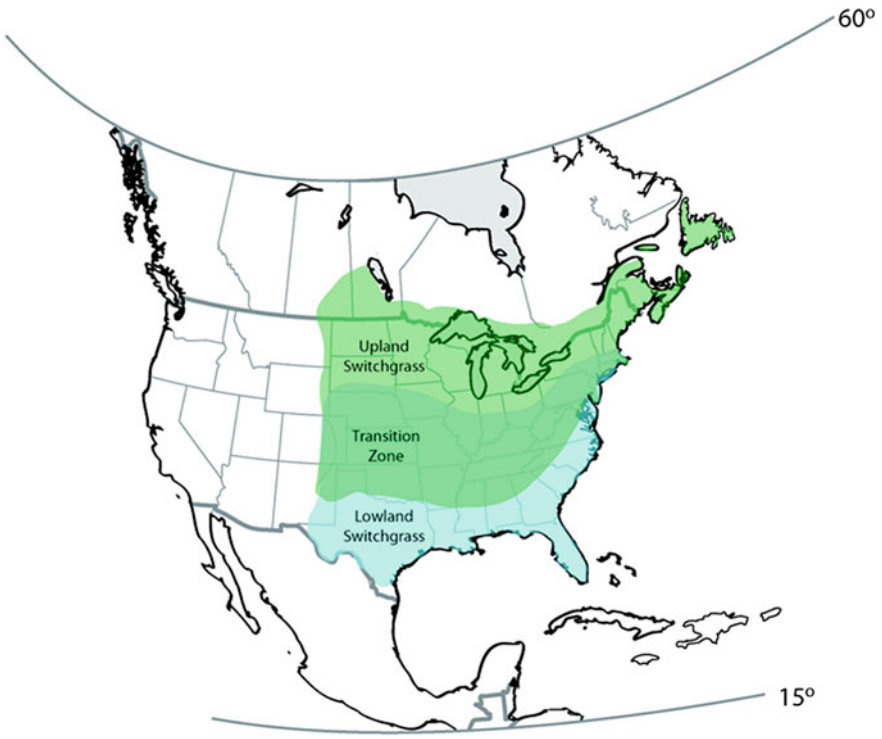

Fig. 2.2 Native ranges of upland and lowland switchgrass ecotypes in North America ([27], reprinted with permission)

Table 2.1 Summary of the most common range of phenotypic values for upland and lowland switchgrass plants grown in direct-comparison experiments in Wisconsin and New Jersey (40–42°N latitude)[a]

Ecotype	Heading date[b] (doy)	Plant height (m)	Flag leaf length (cm)	Flag leaf width (mm)	Number of tillers (# plant^{-1})	Stem diameter (mm)	CIE x-scale color[b]	CIE y-scale color[b]
Upland	180–195	0.9–1.7	32–48	9–11	150–300	3–5	$x < 0.4$	$0.4 < y < 0.8$
Lowland	205–220	1.9–2.2	50–58	12–14	40–90	5–7	$x < 0.2$	$0.2 < y < 0.4$

[a] Cortese et al. 2010 [18]; Casler et al. 2010, unpublished data

[b] Heading date = day of year. Color reference: McLaren [94]; http://www.colorbasics.com/CIESystem/

12–15 major ice age cycles [9] that have compressed the native range of switchgrass into a relatively narrow band along the current coastline of the Gulf of Mexico [10]. Ice ages forced upland and lowland switchgrasses to occupy a relatively narrow region for tens of thousands of years, allowing upland and lowland ecotypes to occasionally mate with each other.

These matings probably occurred at a relatively low frequency, due to differential flowering time, but resulted in significant and measureable gene flow between the two ecotypes [8, 11]. As such, visual or morphometric assessments of plant phenotype are no longer reliable as a mechanism of classifying plants into the upland or lowland ecotype taxa [12]. The most reliable classification method is based on sequence-based marker analysis of plastid DNA, specifically chloroplast DNA, which evolves very slowly over time, much more accurately reflecting the ancient division between the two ecotypes [6–8].

Following each ice age, gradual and punctual warming of the earth's climate resulted in a slow, gradual, and highly discordant northward migration of species to repopulate their former habitats. Because plants are sessile organisms and former habitats became buried under many meters of glacial outwash and sediment, northward migrations of switchgrass and other species required assistance from birds and mammals. Deep sampling of pollen from lakebed sediments has shown that this process required thousands of years and followed a progression of tundra, taiga, boreal forest, deciduous or mixed forest, and grassland. Minor climatic shifts were often sufficient to cause local extinctions and short-term cyclic changes between dominant habitats during the early and mid-Holocene period [13–15]. Because switchgrass and other species were wholly dependent on animals for transport to new sites, it should not be surprising to observe multiple genetic lineages of switchgrass at any particular site; indeed, this is a common occurrence [6, 8, 11, 12].

McMillan [16] proposed that switchgrass was preserved in three refugia during the ice ages, corresponding largely to the eastern Gulf Coast, western Gulf Coast, and western montane (dryland) regions. His research was based on the most extensive collection and evaluation of switchgrass accessions ever conducted, representing nearly the entire range within the USA. Molecular marker analyses of both nuclear and plastid DNA have confirmed that Calvin McMillan was largely correct, but suggest that his "three" refugia might not have been isolated from each other to the extent that he hypothesized. During the Holocene period that followed the Pleistocene glaciation, tallgrass prairie and savanna establishment was largely completed by approximately 2–3,000 year BP. Since that time, climate and animals have continued to mold the genetic landscape of switchgrass, due largely to its highly outcrossing nature and the dominance of wind-aided pollination. Individual accessions of switchgrass, samples of plants collected from a single remnant site, typically possess between 65 and 80% of the genetic variability observable within the species, based on DNA markers [8, 11, 12, 17–22]. Genetic differentiation occurs on a fine-scale in some cases, e.g. changes in soil type or microclimate, but is largely associated with the photoperiod-temperature cline and an east–west cline of humidity and/or precipitation. McMillan hypothesized that the western montane refuge was largely the source of switchgrass genotypes that populated the western portion of its range, where dryland environments and drought tend to be more frequent than in eastern North America. More recent phenotypic studies across a range of sites have confirmed a tendency for eastern accessions to have relatively poor performance at western sites and vice

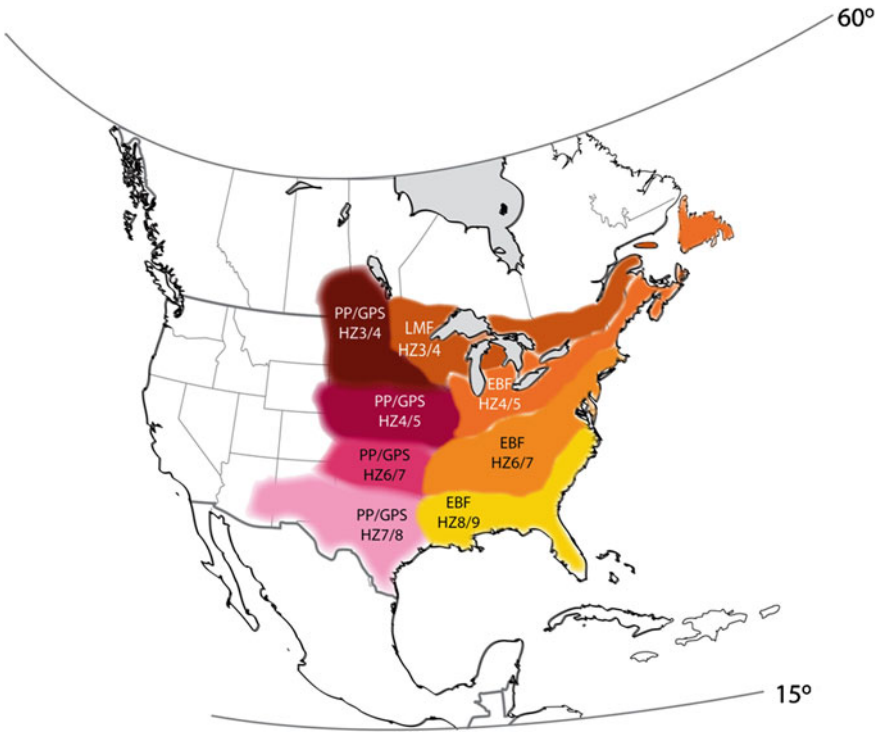

Fig. 2.3 Proposed gene pools for deployment of regionally adapted switchgrass germplasm and cultivars for use in breeding programs or in conservation and restoration projects *PP* prairie parkland, *GPS* great plains steppe, *LMF* laurentian mixed forest, *EBF* eastern broadleaf forest [23]; *HZ* USDA hardiness zone [93] ([92], reprinted with permission)

versa [2]. Anecdotal observations have suggested that eastern accessions lack the drought tolerance to perform well in the western regions, while western accessions lack the disease resistance to perform well in the more humid eastern regions.

One net result of these studies has been the development of a concept of gene pools for switchgrass (Fig. 2.3). Each of the proposed gene pools spans a region that includes two neighboring hardiness zones, with a range in mean temperature of no more than 10°C. The east–west division approximately follows the Mississippi River Valley, splitting the range according to historic tallgrass prairie versus historic savanna ecosystems [23], Sanderson et al. [24] has estimated that cultivar recommendations that follow this regional gene pool concept are responsible for approximately a 20–25% increase in local biomass yields, simply associated with choosing appropriately adapted cultivars. There is currently at least one switchgrass breeder located in each of the eight regions shown in Fig. 2.3, creating opportunities to develop regionally adapted cultivars that take advantage of the significant genotype x environment interactions that are common to this species.

2.3 Genetics and Cytology

Switchgrass has a basic chromosome number of x = 9, with a wide range of chromosome numbers for somatic cells ranging from n = 18 to 108 [25, 26]. Lowland ecotypes of switchgrass are largely tetraploid with 2n = 4x = 36 chromosomes, while octoploids with 2n = 8x = 72 chromosomes are very rare [11]. Conversely, upland ecotypes exist at both tetraploid and octoploid levels, with the octoploid form approximately two to three times more abundant than the tetraploid form. Tetraploids contain approximately 3.1 pg DNA per nucleus with a haploid genome size of ~1.5 Gb [27]. True hexaploids, 2n = 6x = 54, are extremely rare but have been observed within remnant sites that possess both tetraploid and octoploid plants, suggesting their potential role in interploidy gene flow [11]. Recent results based on genotype-by-sequencing suggest that hexaploids likely arise by union of a normal gamete and an unreduced (2n) gamete (Costich et al. 2011, unpublished data). Although 2n gametes are common in grasses [28], they have yet to be verified in switchgrass. Aneuploids appear to be common in switchgrass, particularly at the octoploid level, and largely characterized by chromosome loss from the normal euploid number [29].

Switchgrass is a disomic polyploid, or allopolyploid, with largely diploid inheritance at the tetraploid level [30]. Tetraploid switchgrass has 18 linkage groups arranged in two highly homologous sets that are highly conserved with other C_4 grasses, e.g. *Sorghum* and *Setaria* [30]. Meiosis of both tetraploid and octoploid individuals is largely characterized by normal bivalent pairing [31, 32]. A high frequency of DNA markers are characterized by segregation distortion and multi-locus interactions that could be caused by low frequencies of quadrivalent pairing including homeologous chromosomes [30], which could occur in a recent polyploid such as switchgrass.

Switchgrass is predominantly cross-pollinated with a gametophytic self-incompatibility system similar to the S-Z system found in many grasses [33]. Pollen is dispersed by wind and early reports suggested that the percentage of selfed seed was low in most genotypes, generally <1% [33, 34]. More recent reports suggest that some genotypes are capable of producing selfed seed in frequencies as high as 50% (Buckler et al. 2011, unpublished data; [35]).

Upland and lowland ecotypes of switchgrass can be easily crossed with each other at the tetraploid level, reflecting their relatively recent divergence on the evolutionary scale [33]. A single hybrid between a random upland and a random lowland plant produced a cross with an average of ~35% high-parent heterosis, suggesting that the evolutionary divergence between the two ecotypes has been sufficient to create genetic divergence and allelic complementarity. A post-fertilization incompatibility system between ploidy levels minimizes the opportunity for interploidy crosses and gene flow. The existence of 2n gametes in switchgrass would be an effective mechanism to bridge this barrier. Vogel [36] suggested that tetraploid and octoploid plants that are sympatric within a single remnant prairie site are effectively members of different interbreeding populations.

2.4 Germplasm, Ecotypes, and Early Use

Early cultivars of switchgrass are exclusively represented by seed increases from source-identified remnant prairies (Table 2.2). These natural-track cultivars have generally undergone no direct selection for agronomic traits or been subjected to any plant improvement efforts. Most of these cultivars were given a name that reflects the geographic location of the original accession. Most of these cultivars were collected and evaluated by personnel of the plant materials centers (PMC) of the Soil Conservation Service (SCS), which later became the Natural Resource and Conservation Service (NRCS), an agency of the USDA. Many evaluations included common-garden agronomic evaluations of numerous accessions, allowing personnel to chose only one or two of the best populations for use in each region. There are 26 PMC locations within NRCS, 15 of which have been involved in collection and/or release of switchgrass cultivars using this approach. Because these populations represent little or no breeding history, they represent the natural genetic diversity within specific regions of the switchgrass range. Nevertheless, there are exceptions to this, in which different seed lots of a single cultivar have been shown to have diverged from each other, most likely due to seed increase under different environmental conditions [19].

Natural-track cultivars of switchgrass formed the basis for development of a switchgrass seed industry, shared between a small number of private companies and public organizations such as the Nebraska and South Dakota Crop Improvement Associations. The principal use of switchgrass since the 1940s has been for pasture and rangeland, largely in the Great Plains region of the USA, but only sparsely in the eastern portions of North America. In addition, switchgrass has been a component of seed mixtures for prairie and savanna restoration projects since the mid-twentieth century. Early agronomic trials of unimproved cultivars quickly established the presence of large amounts of ecotypic variation, suggesting that some sense of local adaptation should be used in developing recommendations for the geographic range of individual cultivars [36].

With the choice of switchgrass as a herbaceous model species for the U.S. Department of Energy (DOE) Biofuel Feedstock Development Program (BFDP) in 1992, public and commercial interest in switchgrass rapidly increased. Because the seed industry was largely based on unimproved cultivars that represented source-identified collections, these cultivars came under rapid and high demand as source material for agronomic field trials and demonstration plots for studying conversion of herbaceous biomass into biofuel. This interest led to an expansion of the switchgrass seed industry and the establishment of hundreds of field experiments across the native range of switchgrass in the USA and Canada. Many of these field experiments were highly valuable in helping to define the limits of adaptation of individual cultivars. Even though they were not centrally coordinated or conducted under uniform conditions, these experiments were responsible for identifying Cave-in-Rock and Alamo as cultivars with remarkably broad adaptation. Alamo, from central Texas, can be successfully grown throughout the southeastern USA and along the Atlantic Seaboard into southern New England [37]. Cave-in-Rock is broadly adapted in hardiness zones 4

Table 2.2 Switchgrass cultivars and released germplasm populations representing various habitats in the central and eastern USA, largely representing local ecotypes with minimal or no selection for plant traits

Cultivar	PI number[a]	Ecotype	Ploidy	Year of release	Geographic origin	USDA hardiness zones[b]
Alamo	422006	Lowland	4x	1978	Southern Texas	6, 7, 8, 9
Kanlow	421521	Lowland	4x	1963	Northern Oklahoma	6, 7
Pangburn		Lowland	4x	NA[d]	Arkansas	6, 7
Penn Center		Lowland	NA	2010	Coastal South Carolina	8
Stuart	422001	Lowland	4x	1996	Southern coastal Florida	9, 10
Timber		Lowland	4x	2009	Unknown mixture[e]	6, 7, 8
Miami	421901	Up/Low[c]	4x	1996	Southern Florida	9, 10
Wabasso	422000	Up/Low	4x	1996	Southern coastal Florida	9, 10
Dacotah	537588	Upland	4x	1989	Southern North Dakota	2, 3, 4
Falcon	642190	Upland	4x	1963	New Mexico	4, 5, 6
Grenville	414066	Upland	NA	1940	Northeastern New Mexico	4, 5, 6
High Tide		Upland	NA	2007	Northeastern Maryland	5, 6, 7
KY1625	431575	Upland	4x	1987	Southern West Virginia	5, 6, 7
Blackwell	421520	Upland	8x	1944	Northern Oklahoma	5, 6, 7
Caddo	476297	Upland	8x	1955	Central Oklahoma	6, 7
Carthage	421138	Upland	8x	2006	North Carolina	5, 6, 7
Cave-in-Rock	469228	Upland	8x	1973	Southern Illinois	4, 5, 6, 7
Central Iowa	657600	Upland	NA	2000	Central Iowa	4, 5
Forestburg	478001	Upland	8x	1987	Eastern South Dakota	3, 4
Nebraska 28	477003	Upland	8x	1949	Northeast Nebraska	3, 4
Shelter		Upland	8x	1986	Central West Virginia	4, 5, 6
Southlow	642395	Upland	NA	2003	Southern Michigan	4, 5, 6

[a] GRIN accession number (http://www.ars-grin.gov/). Empty cells indicate that an accession is not available through GRIN; [b] USDA Hardiness Zones are defined in approximately 5°C increments of mean annual minimum temperature (http://www.usna.usda.gov/Hardzone/ushzmap.html); [c] Upland cytoplasm, but lowland phenotype and nuclear DNA, suggesting an ancient hybrid origin [12]; [d] *NA* information not available; [e] DNA marker analyses suggest a mixture of germplasm from the southern Great Plains and the southeastern USA [8, 11]

through 7, covering much of the eastern USA and Canada north of 35°N latitude, but is poorly adapted to dryland regions [38]. Most other cultivars have significantly narrower ranges of adaptation, more aligned with the regional gene pools shown in Fig. 2.3.

Numerous collections of switchgrass have been generated throughout the species range. The official USDA collection of switchgrass accessions is located at Griffin, GA, part of the national plant germplasm system (NPGS) and germplasm resources information network (GRIN). At the time of this writing, the GRIN collection consists of 497 historical accessions, of which 174 are currently available for distribution. A small number of seeds are made available to anyone anywhere, upon request through the web link.[1]

[1] http://www.ars-grin.gov/

There are thousands of additional accessions that are currently being stored in collections made by both public and private organizations involved in restoration, conservation, production, breeding, and genetics. Regardless of where they are housed, these collections are all essentially private, because they are not made broadly available to the public. Any accessions can be donated to GRIN, simply by contacting the switchgrass curator via the GRIN web link. Either seed or living tillers can be donated, but seed is preferred because it requires less urgency for care and handling. Source-identified accessions are preferred and basic passport or descriptive information about the collection site is highly desirable as a link between each accession and its natural environment. Donations can be made of switchgrass germplasm at any stage of development, ranging from wild populations to highly bred cultivars.

2.5 Genetic Improvement

2.5.1 Breeding Objectives

Switchgrass breeding was initiated at the University of Nebraska in the 1950s, largely based on regional seed collections as the basis for breeding populations [39]. Early objectives were focused largely in improving forage quality for livestock production systems, as well as fundamental research to develop a more thorough understanding of reproductive biology and breeding behavior of switchgrass.

Development of a high-throughput in vitro dry matter digestibility (IVDMD) assay [40] was one of the major research breakthroughs that led to some of the most significant breeding gains in switchgrass. Three cycles of selection increased IVDMD by 5% over the original population mean. Simultaneous improvements in both IVDMD and forage yield are possible, but at a reduced rate of gain for both traits due to the reduced individual-trait selection pressure required for multi-trait selection [34, 41–43]. Several improved cultivars have been derived from these efforts to improve forage quality of switchgrass (Table 2.3).

Genetic improvements in forage quality traits such as IVDMD are remarkably stable across environmental conditions and management systems [44, 45]. This principle allowed the rapid cycling of high-IVDMD switchgrasses from breeding nurseries into small-plot trials of agronomic traits and large-plot grazing trials to measure livestock performance. Early grazing trials demonstrated that a 40 g kg^{-1} increase in IVDMD was realized as an increase in daily live-weight gains 0.15 kg animal $unit^{-1}$, an increase in beef cattle production of 67 kg ha^{-1}, and an increase in profit of \$59 ha^{-1}, measured in 1998 \$US [46]. Documentation of improved livestock production, directly associated with increases in IVDMD, was responsible for rapid adoption and success of these cultivars on many thousands of hectares in the years following release of these improved cultivars.

Table 2.3 Improved switchgrass cultivars and germplasm releases representing significant breeding and selection activities

Cultivar	PI number[a]	Ecotype	Ploidy	Year of release	Principal traits selected during cultivar development[b]	USDA hardiness zones[c]
EG2101		Upland	8x	2009	Biomass yield, spring vigor, rust resistance	4, 5, 6
Pathfinder	642192	Upland	8x	1967	Biomass yield and vigor	4, 5
Shawnee	591824	Upland	8x	1996	IVDMD, biomass yield	5, 6, 7
Sunburst	598136	Upland	8x	1998	Large seed size and mass	3, 4, 5
Trailblazer	549094	Upland	8x	1984	IVDMD, biomass yield	4, 5
Summer	642191	Upland	4x	1963	Earliness, rust resistance	4, 5
BoMaster	645256	Lowland	4x	2006	IVDMD, biomass yield	6, 7, 8
Cimarron		Lowland	4x	2008	Biomass yield	6, 7, 8
Colony	658520	Lowland	4x	2009	IVDMD, biomass yield	6, 7, 8
EG1101		Lowland	4x	2009	Biomass yield, spring vigor, rust resistance	8, 9, 10
EG1102		Lowland	4x	2009	Biomass yield, spring vigor, rust resistance	6, 7, 8
Performer	644818	Lowland	4x	2006	IVDMD, biomass yield	6, 7, 8
TEM-LoDorm	636468	Lowland	4x	2007	Reduced post-harvest seed dormancy	6, 7, 8

[a] GRIN accession number (http://www.ars-grin.gov/). Empty cells indicate that a cultivar is not available through GRIN; [b] *IVDMD* in vitro dry matter digestibility; [c] USDA Hardiness Zones are defined in approximately 5°C increments of mean annual minimum temperature (http://www.usna.usda.gov/Hardzone/ushzmap.html)

Increases in IVDMD have been largely associated with reductions in lignin concentration [44] and reduced ratios of p-coumaric/ferulic acid [47]. Low-lignin switchgrass genotypes have also significantly reduced cortical fiber and secondary wall thickenings in stem tissues, compared to high-lignin genotypes [48]. These low-lignin genotypes have significant potential to improve conversion efficiency of switchgrass biomass to bioenergy in a fermentation system where lignin is one of the chief factors limiting ethanol production [49, 50].

Three different approaches have been used in efforts to improve establishment capacity of switchgrass. Selection for large seed size was highly effective in increasing mean seed mass by up to 50% compared to other cultivars, resulting in double the emergence rate and 6-week seedling height [51]. Selection for high seedling shoot mass was highly successful, but did not translate into consistent improvement of seedling vigor, root growth, or establishment capacity in field studies [52]. Finally, divergent selection for elevated versus reduced crown node position failed to affect seedling vigor or establishment capacity [53]. Given the persistent establishment problems associated with switchgrass, especially as a monoculture for bioenergy production systems, there has been surprisingly little research conducted on breeding approaches or directed breeding efforts to improve establishment capacity. Instead, research efforts have largely focused on establishment methods to reduce weed competition (see Chap. 4).

Seed dormancy is a significant problem in many natural populations of switchgrass, but every cycle of selection in a breeding program moves one step closer to reducing seed dormancy problems for improved cultivars, simply by the process of eliminating seeds that do not germinate. Gradual elimination of seed dormancy problems in switchgrass is currently the first of several wild traits to be eliminated on the road to domestication of this species.

A number of pathogens and herbivorous or boring insects utilize switchgrass as a host in various portions of its range [36, 37]. Breeding for resistance to many of these pests is a high priority for most switchgrass breeders, but is largely conducted as a secondary objective in field-based breeding programs. There are no reports of pest screens conducted under highly controlled or uniform conditions of artificial inoculation. Rather, breeders have tended to rely heavily on natural inoculum in field nurseries, which can often be highly variable, inconsistent, and unreliable. Susceptibility to pests is one of the principal sets of traits that are used to eliminate plants from breeding nurseries prior to measurement of traits related to productivity and quality. Genetic variation is known to exist for some pests (e.g. [54]) and disease epiphytotics and insect infestations will likely elevate pest resistances to higher priorities in the future. There are several recent reports of widespread insect pests that are capable of drastically reducing biomass yields and/or seed yields of switchgrass [55, 56]. Smut, caused by *Tilletia maclaganii*, has been reported in several switchgrass fields in Iowa, USA, already causing up to 50% reduction in biomass yield with high infection rates [57].

The global emphasis on development of herbaceous energy crops has led to increased emphasis on biomass yield as the principal breeding objective for most switchgrass breeding programs. Economic studies have clearly indicated that biomass yield of on-farm production systems is the most important factor limiting economic viability of switchgrass as a bioenergy crop [58]. Significant improvements, directly associated with selection and breeding, have been demonstrated in a diverse array of environments and breeding populations [41, 42, 59–61]. Typical rates of gain have averaged about 1–2% year^{-1} and some have been sustained for multiple cycles of selection. Realistically, these results suggest that switchgrass breeders have likely increased biomass yields by an average of 20–30% since the inception of the U.S. DOE BFDP in 1992. Many of these gains are yet to be realized or quantified in agronomic or field-scale demonstration trials due to the 8–10 year lag required to complete evaluations, cultivar releases, and seed multiplications.

While the use of locally adapted and representative germplasm is a cornerstone of restoration and conservation applications for switchgrass, breeders are bounded only by what germplasm will survive or can be modified to survive in their target environments. Switchgrass breeders are beginning to take advantage of southern germplasm, collecting and evaluating germplasm from as far south as possible in an effort to extend the effective growing season. Biomass yield of switchgrass peaks near anthesis [43], often leaving 6–8 weeks of unutilized growing season in northern climates. Lowland ecotypes, which can be 4–6 weeks later than upland ecotypes in flowering time, are currently being selected and bred for improved

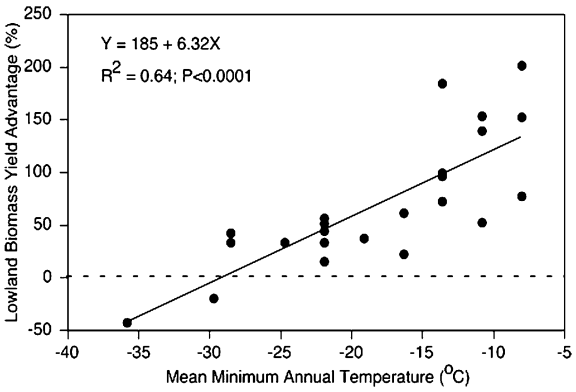

Fig. 2.4 Relationship between lowland-ecotype biomass-yield advantage and mean minimum annual temperature (hardiness zone definitions—[93]) for 23 cultivar-evaluation trials conducted under varying climatic conditions in the USA. Each point is represented by a mean of at least two upland and two lowland cultivars and the difference is expressed as a percentage of the upland mean. Data were collected from [3] (Arlington and Spooner, WI; Mead, NE; Manhattan, KS; Stillwater, OK), [95] (Hope, AR; College Station, Dallas, and Stephenville, TX), [96] (Princeton, KY; Raleigh, NC; Jackson and Knoxville, TN; Blacksburg and Orange, VA; Morgantown, WV), [97] (Chariton, IA); and [4] (Beeville, College Station, Dallas, Stephenville, and Temple, TX)

cold tolerance and biomass yield at northern latitudes. A meta-analysis of 23 field trials conducted in a wide range of climatic conditions shows a strong relationship between lowland-ecotype biomass yield advantage and mean minimum temperature (Fig. 2.4). Lowland cultivars are generally at least twice as productive as upland cultivars at the most southern locations, but this advantage gradually declines with increasing latitude. Lowland cultivars can be expected to have biomass yields 30–50% higher than upland cultivars in the transition zone where both are well adapted. At the most extreme northern locations, biomass yield of lowland cultivars is limited by their inability to survive multiple winters [3].

2.5.2 Breeding Methods

By far the most common breeding method used on switchgrass is some form of phenotypic recurrent selection, mostly using one or more restrictions as proposed by Burton [62] and described in detail by Vogel and Pedersen [63] and Burson [64]. Breeding begins with the assembly of germplasm to be evaluated for inclusion in adapted populations. Nearly all switchgrass breeders conduct initial switchgrass germplasm screens as spaced-plant nurseries at a single location. Because most breeding programs are focused on regional adaptation (Fig. 2.3), a single representative location is typically sufficient to make gains. Spaced plantings are used to conduct efficient evaluations of individual genotypes over 2 or

3 years, allowing each genotype to express itself without competition. Many breeders use some form of mental ideotype in these nurseries, selecting for a range of traits that provide the morphological form desired for the region and the production system. For example, several studies of both upland and lowland ecotypes [65–67] have suggested that tiller density may be the most efficient indirect spaced-plant selection criterion for improving biomass yield.

Although spaced-plant nurseries are generally ineffective toward improving biomass yield of C_3 forage crops [45], they have been highly useful for improving biomass yield of switchgrass. All of the gains cited in the previous section were based on selection in spaced-plant nurseries. Controlling spatial variation within spaced-planted nurseries is critical to ensure a moderate to high heritability for biomass yield. Missaoui et al. [60] accomplished this using a moving-mean or Papadakis-type analysis, similar to nearest-neighbor analysis. Casler [59] controlled spatial variation by conducting all selection within 10-plant blocks, forcing each block to be a mini-selection nursery. Rose et al. [61] evaluated both low- and high-yield environments for selection nurseries and observed greater genetic gain for biomass yield in the low-yield environments.

Spaced plantings are highly efficient for improving quality traits related to feeding value for ruminants or conversion to bioenergy. Traits such as IVDMD and lignin concentration have sufficiently high heritabilities for unreplicated spaced plants that gains can easily exceed 2% $cycle^{-1}$ [44, 68]. Quality traits also possess high genetic correlations between spaced-plant and sward-plot conditions, allowing gains made on spaced plantings to be quickly transferred to real-world pastures and hay fields where performance is measured in terms of livestock production [69, 70].

Duration of spaced plant nurseries can be a critical factor in the success of a breeding program. Reducing cycle time to speed up gains can result in insufficient time in the field to evaluate critical traits such as cold tolerance, heat tolerance, pest resistance, and tiller production. Three cycles of selection for high IVDMD were highly successful, but the third cycle resulted in catastrophic winter injury in the Cycle-4 nursery (<10% survival), largely attributed to selection based 3-year-old plants that had survived only two winters of cold stress [43]. Selection of survivors from this nursery, combined with an additional winter of observation prior to final selection decisions has been attributed to reversing this loss in plant fitness [43].

A typical spaced planting in a switchgrass nursery consists of 1,000–10,000 plants. Once selections are made from a spaced planting, plants are intercrossed to create seed to begin the next cycle of selection. Intercrossing can be conducted in situ leaving the unreplicated plants in place [59], by transplanting selections to an isolated crossing block [43], or by vegetatively propagating the genotypes and transplanting them into a replicated polycross block [63]. If the final data collection and selection decisions are made late in autumn, plants can be transplanted once they are dormant, or transplanting can take place very early in spring without compromising the ability of plants to intercross and produce seed. Some breeding programs intercross selections in the glasshouse, which can be effective following

selection and transplanting from the field at numerous times during the growing season. Switchgrass does not require vernalization to flower, so the most critical factor is to obtain clonal ramets that possess sufficient numbers of tillers or tiller buds to generate inflorescences. Photoperiod adjustment, including low-irradiance 24 h photoperiod, can be used to promote flowering in the glasshouse and to synchronize flowering among genotypes of widely different origins [71].

Family-based selection methods have become commonplace in switchgrass breeding programs. Half-sib families are the most common type of family, largely because they are simple and efficient to produce. Family-based selection methods strive to utilize as much genetic variability as possible by conducting selection among and within families. Both of the USDA-ARS breeding programs in Lincoln, NE and Madison, WI rely heavily on half-sib family selection, using family rows of spaced plants. Both programs attempt to create a competitive environment for individual plants using two different methods. In Lincoln, highly rhizomatous plants are spaced 1.2 m apart, but their spread is constrained to 0.5×0.5 m^2 by frequent tillage [72]. Plants are harvested and biomass yield is expressed on a unit-area basis. In Madison, family rows are created with a plant spacing of 0.3 m within rows and 0.9 m between rows, allowing plants to begin competing with each other in the second year [59]. If half-sib family seeds are produced in sufficient quantity in the field, half-sib family breeding methods can utilize drill-seeded plots to more accurately simulate a realistic agricultural production system [59].

Cycle time for switchgrass breeding programs ranges from 2–7 years, depending on the objectives and specific methods. Theoretically, each cycle could spin off a new and improved population that could move into candidate-cultivar status [63]. Most breeding programs establish new field trials of candidate cultivars every 2–4 years, depending on timing and resources. These field trials are more extensively replicated than selection nurseries, utilizing multiple locations throughout the target region of environments, replicated and randomized experimental designs, drill-pots that can provide accurate biomass yield assessments, and 2–4 years of agronomic-trait measurements. Because many breeders do not have access to a wide array of test sites, some switchgrass breeders collaborate by pooling resources and sharing test sites for candidate-cultivar field trials, particularly when some of the candidate cultivars may have expected adaptation to broader regions than the breeder's range of test sites.

Hybrid breeding is expected to be a significant activity in the future. The evolutionary divergence between upland and lowland ecotypes has been sufficient to create significant allelic differentiation for a wide range of DNA markers and sequences throughout the genome (previously discussed). This allelic diversity has created a certain level of complementarity between uplands and lowlands, such that F1 hybrids have significantly superior performance to their parents [72, 73]. This allelic complementation, manifested as hybrid vigor or heterosis, does not occur in F1 hybrids within either the upland or lowland ecotype. The observation of 30–35% heterosis, superiority of the F1 hybrid to the best of the two parents, lends great optimism to this approach, particularly since the parents were selected more-or-less at random, without any selection for specific combining ability.

Commercial production of F1 switchgrass hybrids is still many years from reality, due to two massive logistical problems. Simply crossing upland and lowland ecotypes with each other is extremely difficult, due to the differences in flowering time of 4–6 weeks (Table 2.1). Flowering time can be relatively easily manipulated in glasshouse or growth chamber environments using temperature, light intensity, and day length [37]. However, commercial hybrids will require field-scale seed production between two clonal genotypes or two inbred lines. Growth regulators, such as the families of compounds that are used to reduce or delay flowering in grasses [74], might be a viable mechanism to promote field-scale interpollination between upland and lowland parents. Field-scale propagation of the parents poses a second problem. Current approaches to F1-hybrid development are based on heterozygous genotypes as parents, requiring some form of efficient and high-throughput vegetative propagation methodology, e.g. somatic embryogenesis or micropropagation [75]. Both parents would be propagated in the laboratory and transplanted in alternate rows to a hybrid-seed production field using existing transplanting technologies (Fig. 2.5). Commercial development will require parents to be screened for specific combining ability (genetic capacity for hybrid vigor in the progeny, complementing the other parent) and competency for large-scale vegetative propagation.

Ideally, and in the long term, such a system should strive to replace heterozygous clonal parents with inbred lines. However, inbred lines are many years from realization in switchgrass, due to self-incompatibility and inbreeding depression. Self-incompatibility is genetically controlled, so it can be modified by selection and breeding for selfing competence. Inbreeding depression can also be partially overcome by long-term selection for vigor and seed production during the inbreeding process, a proven phenomenon in maize breeding, allowing the development of inbred lines capable of seed propagation. This is an extremely long-term goal in switchgrass that is theoretically possible, based on the maize model, but has no precedent in perennial plants.

2.5.3 Impact of Genomics on Breeding

Bi-parental linkage-mapping populations have provided the most definitive evidence that tetraploid switchgrass is characterized primarily by disomic inheritance, acting largely as a diploid organism with 18 pairs of chromosomes [30, 76]. These populations consist of two highly heterozygous genotypes from contrasting populations, crossed under controlled conditions to create a pseudo testcross population. Each parent serves as a tester for markers and quantitative-trait loci (QTL) that are heterozygous in the other parent. Second-generation linkage populations, which would allow detection of markers and QTL that are homozygous for different alleles in the two parents, have yet to be reported in switchgrass. The 18 linkage groups of switchgrass consist of two highly homologous sets of nine chromosomes that are highly collinear with *Sorghum* and *Setaria* [30].

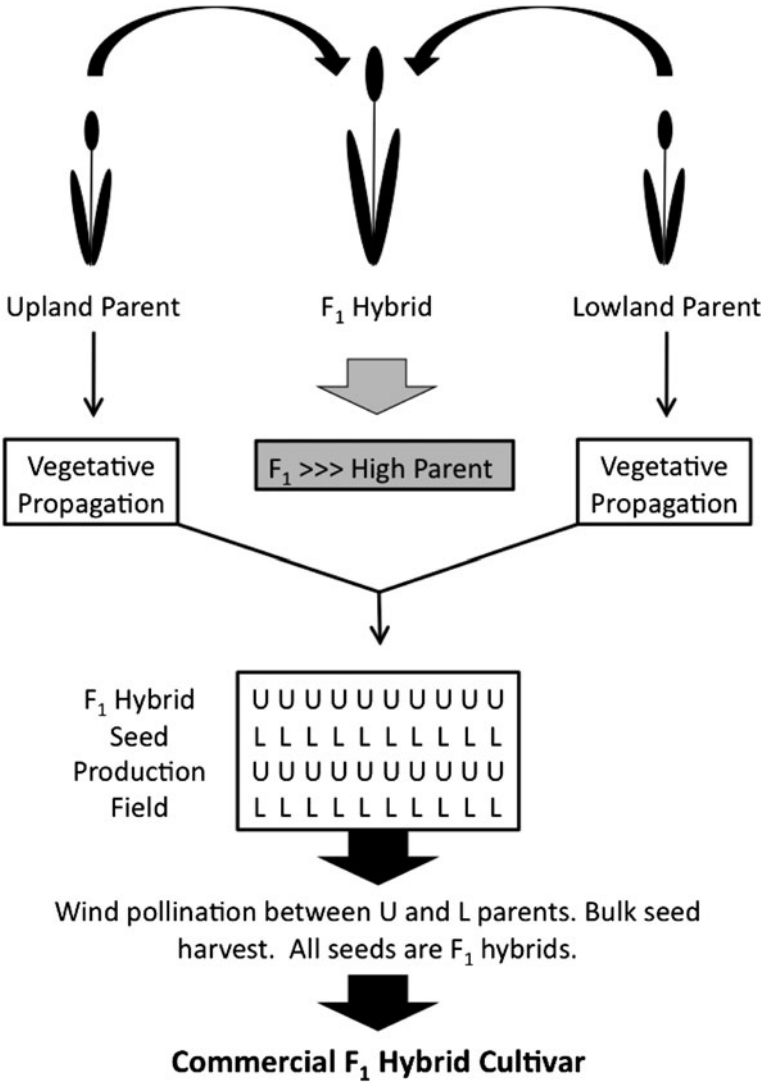

Fig. 2.5 Seed production scheme to develop F_1 hybrids between vegetatively propagated genotypes of upland and lowland switchgrass ecotypes

Numerous DNA marker systems have been adapted for use in phylogenetic studies of switchgrass and development of DNA markers that can be used in switchgrass breeding [27]. Based on these marker systems, both wild switchgrass populations and bred cultivars contain levels of variability equivalent to 65–85% of that found across the range of the species. The remaining variability is associated with the two major taxa (upland and lowland), geographic differentiation

[8, 12], and fine-scale differentiation due to natural selection [6]. The consistent similarity of wild populations and bred cultivars indicates that the limited number of generations of selection in breeding current switchgrass cultivars has not created significant bottlenecks to impact the overall genetic diversity within the cultivars. Selection and breeding has likely impacted a relatively small number of genes scattered throughout the switchgrass genome, preserving genetic variability at the genomic level.

A reference genome sequence is not available for switchgrass, largely due to the complexity and expense involved in sequence assembly of such a complex polyploid. Current efforts by DOE Joint Genome Institute (JGI), Walnut Creek, CA are focused on deep sequencing the parents and 192 progeny from the AP13 × VS16 (Alamo × Summer) bi-parental cross. An existing linkage map of this population will be used to localize genomic scaffolds to one of the 18 linkage groups, creating a reference map that can be used to order and localize future sequence data from other genomic resources of switchgrass, including bacterial artificial chromosomes (BAC, [77]), expressed sequence tags (EST, [78]), and exome sequences that are currently under development [27].

Marker-trait associations have yet to be specifically identified in switchgrass. The AP13 × VS16 bi-parental cross has undergone phenotypic evaluation for several morphological and agronomic traits in southern Oklahoma, but data have yet to be analyzed at the time of this writing. Two association panels of switchgrass were assembled and established in field studies. A northern panel, consisting largely of upland accessions, consists of 10 genotypes each of 60 populations and has been evaluated for single nucleotide polymorphic (SNP) markers and phenotypic traits at Ithaca, NY. A southern panel, consisting largely of lowland accessions, consists of 10 genotypes each of 48 populations and has been evaluated for phenotypic traits at Athens, GA and Ardmore, OK. Future plans will include genome-wide association studies (GWAS) of marker-trait associations within each panel and across the two panels [27].

Finally, genomic selection (GS) offers considerable potential to increase the rate of gain for important traits such as biomass yield [79]. Genomic selection offers two mechanisms to increase selection efficiency and rate of gain. Once a training population of genotypes and families is established, predictive equations are developed and validated to predict phenotype from genotype. This predictive equation can be applied to seedlings to apply indirect selection pressure to important agronomic traits prior to establishment of field-based nurseries, dramatically increasing the proportion of genetic variation utilized in selection [80]. Second, recurrent selection could be altered to eliminate the field-based evaluation in some cycles, relying on short-term maintenance of linkage disequilibrium in the population following only one or two recombination events. For example, Cycle 1 could consist of a field evaluation of biomass yield, equation development and validation, and within-family selection. Cycle 2 (and possibly Cycle 3) could proceed with simple phenotypic selection using the predicted breeding values based on seedling DNA marker analyses, prior to returning to the field for another cycle of field evaluation and recalibration of the predictive equations. Seedling

selection offers huge time advantages because it can be accomplished within 1 year, as opposed to a minimum of 5 years for a typical field-based selection cycle. Validation and computer simulation studies will be critical in determining the number of seedling selection cycles, if any, that can be utilized to speed up the selection process.

Current efforts in Wisconsin USA are focused on development of three training populations: an upland population selected for biomass yield, a lowland population selected for winter survival, and a hybrid population. All populations are structured as half-sib families and the basic selection method will involve selection among families for field-based biomass yield in plots replicated across locations and years and selection of seedlings within families for breeding values predicted from DNA markers. If the accuracy of breeding-value prediction is >0.3, GS with 10% within-family selection intensity has >35% higher expected gains than any phenotype-based selection protocol [80]. If genotyping costs are sufficiently low to increase within-family selection intensity to 1%, expected gains for GS are 70% greater than phenotypic selection.

2.5.4 Genetic Engineering and Risk Assessment

Switchgrass can be transformed with the addition of specific functional genes from other organisms using one of two methods. *Agrobacterium*-mediated transformation uses a biological vector whereas particle bombardment uses a non-biological vector to insert new genes into the switchgrass genome [81]. *Agrobacterium*-mediated transformation tends to result in lower copy number and fewer genomic rearrangements than particle bombardment [82–84]. While efficient, high-throughput genetic transformation systems have been developed for switchgrass, these systems are currently genotype-dependent. Certain genotypes are more responsive to both the tissue culture and transformation phases of this process (Fu and ZY Wang, 2011, personal communication), creating a need for more genotype-independent methodologies.

Genetic transformation of switchgrass to reduce recalcitrance of biomass for conversion to energy has received the greatest amount of attention. Manipulation of one gene is sufficient to create measurable and significant changes to cell-wall composition and structure, impacting sugar release and downstream processing of biomass to energy [85–87]. Reductions in lignin concentration or modifications to lignin structure can positively impact the availability of cell-wall carbohydrates in a fermentation system to produce liquid fuels and in a livestock-production system where switchgrass is a livestock feed [50]. Such changes not only increase energy that is available to microorganisms that conduct fermentation, but also create opportunities to significantly reduce input costs of production, allowing reduced pretreatment severity and enzyme requirements in the case of biofuel fermentation systems [85].

Genetically modified (GM) organisms have traditionally been highly regulated, requiring several years of extensive testing and risk assessment studies [88]. Scientific issues that have potential to impact the regulatory process include the effects of transgenes on plant fitness under a wide range of environmental conditions and their potential to be transmitted to non-transgenic switchgrass. In the case of switchgrass, transmission can occur via either pollen or seed. Pollen-flow studies have not been conducted on switchgrass, but pollen has been known to travel as far as 21 km in *Agrostis* [89]. Seed is literally unbounded in its ability to travel across the landscape, facilitated by birds, mammals, and humans [8]. Risk assessment is further complicated by the need for most assessments to be designed specifically for the gene in question (related to its function in the plant and both proposed and unintended potential functions in the environment), the biology of the species, the likelihood and frequency with which natural stands of compatible relatives exist within the agricultural range of the species, and the range of environmental conditions under which the species will be utilized [90].

Based on its broad natural range, the existence of thousands of native prairie and savanna remnants, and the range of management systems and environmental conditions under which switchgrass can be grown for biomass or forage, deregulation of any switchgrass transgene is guaranteed to result in its dissemination and introduction into natural populations. Switchgrass is a wild plant that still contains many traits common to wild species, including seed dormancy, seed dispersal by shattering, variability in flowering time within individual panicles and plants, and small seeds that can easily escape containment, each of which can contribute to switchgrass seed dispersal and viability over space and time. The scientific community is highly dichotomous over the potential implications of genetic improvement of switchgrass, the agricultural community generally in favor of speeding up domestication and incorporating useful traits (e.g. [85]) and the ecological community more cautious and concerned about limiting impacts outside of agricultural fields (e.g. [91]). In the USA, genetically modified switchgrass is regulated by the Animal and Plant Health Inspection Service (APHIS) of the USDA. APHIS is currently experiencing pressure from the U.S. DOE which has invested millions of dollars into development of new GM concepts and technologies, aimed at improving production and processing of switchgrass biomass for energy production.

2.6 Conclusions

Genetic resources of switchgrass are vast and untapped. Modern cultivars represent no more than five or six cycles of selection removed from wild germplasm, insufficient to create significant genetic bottlenecks. Rates of gain have historically been modest, largely focused on one or two principal traits and likely based on relatively low numbers of genes. As such, switchgrass is still an undomesticated plant with vast potential for improvement of agronomic and biofuel traits.

Huge stores of genetic variability exist for adaptive traits such as pest resistances, stress tolerances, biomass yield and quality traits, and phenological traits, providing a basis for utilizing genetic resources from a broad geographic area to generate highly targeted improvements within regions suitable for biomass production. Genomic resources are rapidly being developed to create opportunities for increasing selection efficiency and rate of gain. Development of a formal switchgrass research community, already underway in 2010, is expected to improve communications among researchers of diverse interests and disciplines and provide mechanisms for researchers to keep up with rapidly developing and changing technologies.

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