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Registration of 'Liberty' Switchgrass

K. P. Vogel,* R. B. Mitchell, M. D. Casler, and G. Sarath

ABSTRACT

'Liberty' (Reg. No. CV-271, PI 669371) switchgrass (*Panicum virgatum* L.) is a lowland-type cultivar that is adapted to USDA plant hardiness zones (HZ) 4, 5, and 6 in the U.S. Great Plains and Midwest, east of 100° W. longitude. It was developed for use as a perennial biomass energy crop and is the first high-yielding biomass-type lowland cultivar adapted to this region. It can produce greater biomass yields than upland- or forage-type switchgrass cultivars developed previously for use in the region, and it has equivalent winter survival. Liberty has significantly greater winter survival in its adaptation region than previously released lowland switchgrass cultivars such as 'Kanlow' and 'Alamo' that frequently have substantial winter damage and stand loss north of 40° N latitude in the U.S. Great Plains and Midwest.

SWITCHGRASS (*Panicum virgatum* L.) is a North American prairie grass that has been used for more than 70 yr in the Great Plains and the Midwest regions of the United States as a warm-season pasture grass (Vogel, 2004), and it is currently under development as a perennial biomass energy crop (Vogel et al., 2011; Casler et al., 2012). The reproductive system of switchgrass determines the breeding systems that can be used to develop new cultivars (Vogel and Pedersen, 1993). Switchgrass has two main ploidy levels, tetraploid ($2n = 4x = 36$) and octoploid ($2n = 8x = 72$); both ploidy levels have bivalent pairing (Vogel, 2004). Switchgrass plants are highly self-incompatible due to an *S-Z* incompatibility system and have a postfertilization incompatibility system that inhibits the production of viable seed when switchgrass plants with different ploidy levels are mated (Vogel, 2004). Pollen is dispersed by wind. Switchgrass has two cytoplasm types, "L" and "U," based on chloroplast DNA (cpDNA) polymorphisms that are associated with the lowland and upland ecotypes, respectively (Hultquist et al., 1996; Vogel, 2004). There are two main groups of ecotypes: lowland types, which are typically found in river bottom lands; and upland types, which are found in natural grasslands not subject to flooding (Vogel, 2004; Casler et al., 2012). All lowland ecotypes are tetraploids, whereas upland ecotypes can be tetraploids or octoploids (Vogel, 2004; Casler et al., 2012). Because of the switchgrass reproductive system, population breeding methods have been the primary breeding procedures used to develop previous cultivars (Vogel, 2004).

The cultivars released to date for use in the Midwest have been upland ecotypes developed and released for use in pastures and for conservation (Alderson and Sharp, 1994; Vogel, 2004; Casler et al., 2012). Switchgrass cultivars for the northern half of the United States have been limited to upland ecotype switchgrass cultivars because available lowland cultivars are from southern latitudes and have poor winter survival in the region (Casler et al., 2004, 2007). Lowland switchgrass cultivars have the potential to produce greater biomass yields if they had better

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Abbreviations: ARDC, Agricultural Research and Development Center; HZ, plant hardiness zone; NIRS, near-infrared spectroscopy.

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winter survival. The native range of switchgrass includes USDA plant hardiness zones (HZ) (USDA, 2012) 3 to 9, which range from southern Canada to Baja California, Florida, and central Mexico (Vogel, 2004; Casler et al., 2012). If existing lowland cultivars based on southern ecotypes are moved too far north, they stay vegetative too late in the growing season and have poor winter survival (Vogel, 2004; Casler et al., 2004, 2007). The objective of the breeding work that resulted in the development of 'Liberty' (Reg. No. CV-271, PI 669371) switchgrass was to develop a high-yielding lowland-type switchgrass cultivar adapted to the Midwest and adjacent areas, where it can be grown as a biomass energy crop. Secondary selection criteria were visual selection for seedling vigor 6 wk after planting in the greenhouse and reduced biomass acid detergent lignin concentration for potential improved conversion efficiency to liquid fuels.

Materials and Methods

All selection, polycross, and seed increase nurseries used in the development of Liberty were located at the University of Nebraska's Agricultural Research and Development Center (ARDC) located near Mead and Ithaca, NE (41°09' N, 96°25' W). In the space-transplanted polycross and selection nurseries described in this report, rows and plants within rows were spaced on 1.1-m centers, and the following cultural practices were used. Space-transplanted nurseries were established by transplanting greenhouse-grown seedlings into the selection nurseries. The nurseries were cultivated between plants with 0.6-m-wide rototillers creating 0.4-m by 0.4-m miniplots for individual plants. Nurseries were fertilized annually with 112 kg ha⁻¹ N, and herbicides and hand weeding were used for weed control. Space-transplanted nurseries were mowed or burned each spring to remove the accumulated biomass from the previous year.

Breeding History

Liberty was developed using population hybridization and multigeneration within population breeding procedures. The self-incompatibility system of switchgrass allows controlled crosses to be made by bagging panicles from two different plants with the same ploidy level and allowing the plants to intermate, which we refer to as *paired-plant crossing*. Plants of two switchgrass cultivars, 'Summer' and 'Kanlow', were mated using paired-plant crossing in the greenhouse in 1996. Summer is a tetraploid, upland cultivar that is based on germplasm collected in southeast Nebraska, and Kanlow is a tetraploid, lowland cultivar that originated from a collection in Oklahoma.

Seed harvested from 13 Summer plants in the paired-plant matings was used to grow Kanlow × Summer F₁ plants, which were transplanted into field evaluation nurseries at the University of Nebraska's ARDC in spring 1997. Some of the seedlings were used in a genetic study (Martinez-Reyna and Vogel, 2008). The remaining 172 seedlings were transplanted into a space-transplanted holding and evaluation nursery. In 1999, 51 plants were selected for vigor and survival from the 172 plant space-transplanted nursery, and two ramets of each plant were transplanted at random into an isolated polycross nursery that was used to produce Syn-2 seed. The Syn-2 seed was harvested in bulk from all plants in the polycross nursery. The

Syn-2 seed was used to establish an isolated generation advance nursery using 600 transplanted seedlings that produced Syn-3 seed as mature plants. The two generations without selection were used to stabilize the population.

The Syn-3 seed was used to grow 2900 seedlings in containers or mini-pots in the greenhouse. After seedlings had emerged, they were thinned to one per cell. Six weeks after emergence, the 20 most vigorous seedlings within each of 30 trays of 98 seedlings were selected for transplanting into a field selection nursery at the University of Nebraska's ARDC in spring 2002. The selection nursery was a space-transplanted nursery with 30 rows of 20 plants on 1.1-m centers and was managed as described previously. No data were collected in 2002 or 2003. In mid-August 2004, when plants in the selection nursery were at the R2 stage of development (panicles emerged from the boot; Moore et al., 1991), about one-third of the plants in a row (10 plants) were visually selected per row for biomass harvest for yield. Before harvest, approximately five whole tillers were hand harvested from each plant selected for harvest and placed in kraft paper bags for drying and subsequent separation into plant parts. The samples were weighed before and after drying to determine dry matter concentration. After sampling, the individual plants were harvested with a Carter Forage Harvester (Carter Manufacturing Co.) with a cutting height of 10 cm and harvested yields determined. A total of 200 plants were harvested and sampled. The plant samples collected from each harvested plant were hand-separated into stem, sheath, leaf, and panicles. The samples were dried and ground sequentially with a Wiley mill (Thomas Scientific) with a 2-mm screen and then with a UDY mill (Thomas Scientific) with a 1-mm screen. Each set of samples, stem, sheath, leaf, and panicles from this experiment as well as other plant part samples from the similar switchgrass selection nurseries was scanned using a NIR System 6500 near-infrared spectroscopy (NIRS) unit (Foss NIRSystems, Inc.). A NIRS calibration set of samples for each sample type that was representative of the NIRS spectra of all sampled plants was analyzed in duplicate for neutral detergent fiber, acid detergent fiber, and acid detergent lignin (ADL) with the ANKOM Fiber Analyzer (ANKOM Technology Corp.) using the procedures described by Vogel et al. (1999) and the ANKOM ADL procedure (ANKOM Technology) Method 9-99 ("Determining Acid Detergent Lignin in Beakers"). Laboratory means were used to develop NIRS calibration equations by partial least squares (Shenk and Westerhaus, 1991). A project-developed selection index was used to select plants for high biomass dry weight yield (YLDDM) and low stem lignin concentration (STLIG) with both traits receiving equal weight. The selection index (NETO2) was used because high biomass yields are needed to obtain high yield fuel yields per acre and low stem lignin concentration can improve ethanol yields per tonne in a cellulosic biorefinery (Dien et al., 2006). The index was calculated using the following equation:

$$\text{NETO2} = \frac{[\text{YLDDM} - \text{YLDDM Mean}]/\text{YLDDM SD}}{+ [-1 * (\text{STLIG} - \text{STLIG Mean})/\text{STLIG SD}]}$$

Thirty-four selected plants were transplanted in an isolated polycross nursery in 2006 to produce Liberty Syn-1 seed, which was evaluated as the experimental strain Kanlow × Summer

HP1 NETO2 C1. Isolated polycross nurseries in the switchgrass breeding at Lincoln, NE, are typically separated from other switchgrass plants by 400 m.

Evaluation Trials

Sward Trials

Multiyear biomass yield sward evaluation trials were conducted at locations near Mead, NE (HZ 5), Dekalb, IL (HZ 5), and Marshfield (HZ 4), Arlington (HZ 4), and Spooner, WI (HZ 3) during the period 2008 through 2011 in which cultivars and experimental strains including Kanlow × Summer HP1 NETO2 C1 were evaluated for biomass yield, plant survival, and other traits. Other experimental strains based on Kanlow breeding populations as well as Summer × Kanlow (female) and Kanlow × Summer (female) lowland × upland hybrid populations were included in these trials (Table 1, 2). The plots at Mead, NE, were 1.2 by 3 m and were planted with a small plot drill using a seeding rate of 400 pure live seeds m⁻². The plots

for the Wisconsin and Illinois trials were 0.9 by 1.8 m planted with a small plot drill at a similar seeding rate. Each experiment was a randomized complete block with four replicates, except the Mead ARDC trial, which had 12 replicates. The breeding background of the other lowland experimental strains and the upland experimental strains from both the ARS Lincoln and ARS Madison, WI, switchgrass breeding projects that were included in the trials were described by Casler and Vogel (2014). Small plot trials were managed to maximize biomass yields and were harvested after killing frost in 2009, 2010, and 2011. Liberty responded well to existing switchgrass best management practices including quinclorac (3,7-dichloro-8-quinolinecarboxylic acid) herbicide at establishment (Mitchell et al., 2010). Stands in the sward trials were monitored annually during the duration of the trials by using a frequency grid (Vogel and Masters, 2001) after spring green-up. Biomass yields were harvested using small plot flail harvesters at all locations with a cutting height of 10 cm.

Table 1. Biomass yield and stand frequency of occurrence for switchgrass cultivars and experimental strains evaluated near Mead, NE, in sward plots for biomass production. The nursery was established in 2008 and harvested for biomass yield in 2009, 2010, and 2011 after a killing frost.

Strain	Yield	Stand	Stand
	3-yr mean	2009	2011
	Mg ha ⁻¹	%	
Liberty (K×S HP1 C1 High NETO2)†	18.1	70	87
Kanlow	18.7	52	66
Shawnee	12.5	80	83
Trailblazer	10.3	70	57
NE Summer	13.0	84	88
Kanlow N1 Early mat.-HYLD	18.2	58	79
CIR HYD-HDMD C3	13.4	80	85
Kanlow N1 Late mat.-High yield	19.9	62	75
NE 2000 C1 Syn-2	11.7	73	65
Kanlow N1 NETO2	20.2	62	77
NE Late Syn HYLD-HDMD C4 Syn-2	13.1	66	69
Kanlow N1 NETO3	18.9	56	74
K×S HP1 C1 HYLD	18.3	56	78
S×K HP1 C1 HYLD	18.1	75	80
S×K HP1 C1 High NETO2	18.6	69	83
NE Summer Late mat.-High vigor	12.9	82	77
NE Summer Elite Late mat.	13.2	77	74
Kanlow N1 Syn-2	18.7	53	79
Pathfinder	10.7	70	54
St. Croix	11.0	48	59
WS4U-C1	11.9	73	70
WS4U-G1R	11.8	65	83
WS4U-C2	12.4	71	73
WS4U-G2R	11.9	68	88
WS4U-C0	12.0	65	71
WS4U-C3	12.6	78	83
WS4U-G3R	12.2	75	81
WS4U-N1m	12.3	74	78
WS4U-N1a	11.9	67	76
WS4U-N2m	11.3	66	67
WS4U-N2a	12.0	70	72
F statistic for strains	24.36**	3.34**	3.78**
LSD (0.05)	1.0	14	12

** Significant at the 0.01 probability level.

† K, Kanlow; S, Summer.

Individual Plant Survival Trials

Survival information on individual plants from the Kanlow × Summer and Summer × Kanlow cycle 2 selection nurseries (same generation as Liberty) and also from Kanlow-derived populations was obtained using data collected from a set of selection nurseries. The cycle 2 (C2) half-sib family selection nurseries used in the survival studies were produced using seed from polycrossed plants selected in breeding cycle 1. Half-sib family and check cultivar individual plant survival scores for switchgrass lowland (Kanlow and Kanlow-derived strains), upland (Summer), and lowland × upland (Kanlow × Summer) advanced generation hybrid populations in their respective selection nurseries located in the same field at the University of Nebraska's ARDC were determined following a winter in which significant winter injury and plant death occurred. The half-sib family plots or check cultivar plots of

10 plants spaced on 1.1-m centers were transplanted in late spring or early summer of 2007 using greenhouse-grown seedlings. The well-established nurseries were harvested after frost on a plot basis in late October or early November in 2008. The winter of 2008–2009 resulted in significant winter damage to plants in the nurseries, which were all in the same field. In late spring 2009, every plant in the nursery was scored for percentage survival by visually estimating the percentage of its crown that had produced new shoots. Family or cultivar plot means of the 10 plants scored within each plot were used in the analyses of variance to test for differences among families and check cultivars.

Table 2. Mean biomass yields and groundcover percentages of switchgrass cultivars and experimental strains grown at DeKalb, IL, and Arlington, Marshfield, and Spooner, WI. Trials were established in 2008 and harvested in 2009, 2010, and 2011.

Cultivar or experimental strain	Biomass yield				2011 Groundcover			
	DeKalb	Arlington	Marshfield	Spooner	DeKalb	Arlington	Marshfield	Spooner
	Mg ha ⁻¹				%			
Liberty (K×S HP1 C1 High NETO2)†	16.4	9.1	9.1	12.5	93	97	93	100
Kanlow	9.2	4.6	3.2	2.5	82	30	56	10
Shawnee	12.9	12.8	11.1	9.5	93	100	92	100
Blackwell	10.4	9.1	7.8	7.6	97	93	74	100
Carthage	11.9	13.6	8.6	8.3	94	91	87	100
Cave-in-Rock	10.4	13.4	8.4	10.0	96	97	97	100
Forestburg	8.9	7.1	6.2	6.3	98	97	91	100
Pathfinder	8.5	8.3	6.8	8.6	90	89	86	100
SB #1	8.5	6.2	5.2	5.6	92	75	82	100
Summer	8.5	7.2	8.3	9.2	92	82	92	100
Sunburst	8.4	8.2	9.0	6.6	96	99	94	100
Trailblazer	9.3	6.2	4.7	7.7	93	79	62	97
WS8U	11.7	12.7	12.3	10.6	94	99	94	100
CIR HYD-HDMD C3	11.8	13.0	12.6	10.3	93	96	98	100
NE Late Syn HYLD-HDMD C4 Syn-2	10.6	8.0	7.3	8.6	88	87	84	100
NE Summer Late mat.-High vigor	10.4	7.6	8.5	9.5	95	77	93	97
Kanlow N1 NETO3	12.4	4.2	3.8	3.1	89	48	74	7
Kanlow N1 Early mat.-HYLD	10.6	6.2	3.0	1.5	87	44	76	3
Kanlow N1 Late mat.-HYLD	12.6	5.6	5.5	2.5	90	68	67	12
Kanlow N1 NETO2	13.0	5.6	4.2	2.9	91	66	79	18
Kanlow N1 Syn-2	10.5	5.0	3.6	2.8	89	46	63	3
K×S HP1 C1 HYLD	13.0	9.2	10.7	12.8	91	95	91	100
S×K HP1 C1 High yield PC	12.9	10.9	10.7	12.9	94	97	93	100
WS4U-C0	10.0	7.3	8.5	8.5	93	88	94	100
WS4U-G1R	8.9	8.0	9.7	8.0	94	94	94	100
WS4U-G2R	8.6	5.1	8.0	8.7	92	66	87	100
WS4U-G3R	8.6	6.6	7.2	8.6	89	92	92	100
WS4U-C0	10.0	7.3	8.5	8.5	93	88	94	100
WS4U-C1	10.1	8.6	10.9	8.3	95	90	89	100
WS4U-C2	9.8	9.5	10.8	9.1	96	94	91	100
WS4U-C3	11.7	10.2	11.5	9.8	94	89	92	100
WS4U-N1a	10.1	7.1	8.6	7.9	96	83	93	100
WS4U-N1m	9.5	7.6	7.4	7.9	98	88	95	100
WS4U-N2a	9.9	8.1	9.0	7.6	97	93	92	100
WS4U-N2m	9.7	6.0	8.7	8.8	93	77	93	98
LSD (0.05)	1.8	1.7	1.7	1.4	4	11	9	8

† K, Kanlow; S, Summer.

Characteristics

Agronomic and Botanical Description

Liberty has the typical lowland switchgrass phenotype but because the female parent plants in the originating crosses was the upland cultivar Summer, it has upland cytoplasm that was identified using chloroplast DNA markers as described by Hultquist et al. (1996) with minor modifications. Switchgrass maturity, as measured by heading date, can vary with year and environment. The R2 stage of development (Moore et al., 1991), when the panicles have emerged from the boot, was used as the heading date. Liberty is typically at the R2 stage in mid-August at Mead, NE, and is 2 to 3 wk earlier in maturity than Kanlow, its lowland male parent cultivar. Liberty can be distinguished from Kanlow and other switchgrass lowland cultivars by its earlier maturity and chloroplast DNA. It is 10 to 14 d later in maturity than Summer, its female parent cultivar, and can be distinguished from Summer and other upland cultivars by its lowland phenotype. Typical of switchgrass cultivars produced by polycrossing selected plants, there is about a 2-wk range in tiller flowering both within and among Liberty plants. It is a tetraploid cultivar.

Biomass Yield and Stands

Biomass yields of Liberty were 40 and 25% greater, respectively, at Mead, NE (Table 1), and Dekalb, IL (Table 2) (both HZ 5 locations), than the best-available adapted upland cultivars, including ‘Shawnee’, averaged over 3 yr of field trials. At Dekalb, Liberty’s biomass yields were also 25% greater than

Kanlow or any other tested lowland switchgrass experimental strain. Some lowland experimental strains at Mead had greater biomass yields than Liberty but had significant stand loss in space-planted evaluation nurseries during the winter of 2008–2009, while plants in the Liberty population had negligible winter damage (Table 3). At Spooner, WI (HZ 3; 45.8° N), Liberty had greater biomass yield than any released cultivar in the trial and maintained 100% groundcover stands during a period in which Kanlow and Kanlow-derived experimental strains had significant winter kill resulting in groundcover scores of <20% (Table 2). Liberty also maintained excellent stands at both Marshfield and Arlington, WI, where Kanlow and Kanlow-derived experimental strains had significant winter damage. At Arlington and Marshfield, Liberty had equivalent or greater biomass yield than switchgrass HZ 4 cultivars that have been released to date, but it had lower biomass yields than Shawnee and some upland experimental strains. Although it had lower yields than some upland cultivars and experimental strains at these two locations, it has the genetic potential to produce greater biomass yields across the Midwest than do the upland switchgrass strains and released cultivars. Its primary merit for release is its ability to maintain stands and produce high yields when managed as a biomass energy crop in the midwestern United States.

Table 3. Half-sib family and check cultivar individual plant survival scores for switchgrass lowland (Kanlow [K] and Kanlow-derived strains), upland (Summer [S]), and lowland × upland (K × S) advanced generation hybrid populations in their respective cycle 2 selection nurseries at the University of Nebraska’s Agricultural Research and Development Center, 50 km west of Omaha, NE.

Breeding population nursery	Family or check	Family number	Reps†	Survival			ANOVA F test	LSD (0.05)
				Mean	Range			
					Low	High		
		<i>n</i>	<i>n</i>	%				
Liberty (K×S HP1 NETO2 C1)	Families	34	5	89	82	95	12.33**	7
	Kanlow N1			43				
	Summer			99				
S×K HP1 HYLD‡	Families	34	4	64	5	77	3.60**	15
	Kanlow N1			26				
	Summer			85				
S×K HP1 NETO2	Families	34	4	58	48	65	7.42**	9
	Kanlow N1			18				
	Summer			79				
Kanlow N1 Late mat.-high yield	Families	30	4	41	18	54	1.36 ns	ns
	Kanlow N1			44				
Kanlow N1 Early mat.-high yield	Families	15	8	40	21	48	8.94**	11
	Kanlow N1			35				
	Summer			84				
Kanlow N1 NETO2	Families	23	5	41	29	64	1.85*	15
	Kanlow N1			33				
Kanlow N1 NETO3	Families	27	5	38	25	54	2.0*	15
Kanlow N1	Kanlow N1			37				

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† The number of plants evaluated per half-sib family or check strain is replication (Rep) number (*n*) × 10.

‡ The female or seed parent for the Kanlow (K) and Summer (S) hybrid populations is listed second in the strain designation.

Discussion

Recommended Areas of Use

Liberty is adapted to USDA HZs (USDA, 2012) 4, 5, and 6 in the U.S. Great Plains and Midwest east of 100° W. longitude and potentially other regions where it has not been tested to date. It has been successfully grown as far north as Spooner, WI (45.8° N). It has been grown on both upland and poorly drained sites at the ARDC, Mead, NE. Seed harvest of Liberty at Mead typically occurs at the end of September or early October, and it likely can be grown for seed at other HZ 5 and 6 locations. Seed production in HZ 4 locations could be adversely affected by early frost damage in many years, which could lead to genetic shifts in the cultivar and is not allowed for certification.

Seed Availability

Liberty is a stable, improved random-mated population and will be maintained and increased accordingly. Liberty was officially released by the USDA-ARS and the Agricultural Research Division of the University of Nebraska on 27 Nov. 2013. Breeder seed will be jointly maintained and produced by the USDA-ARS and the University of Nebraska-Lincoln with random-mated, isolated increase fields originating from Syn-1 breeder seed. Foundation seed production of Liberty will be managed by Husker Genetics, the Foundation Seed Division of the University of Nebraska-Lincoln. Foundation seed will be made available for certified seed production on a nonexclusive basis to seed producers who contractually agree to produce and market the seed only as certified seed using the cultivar name Liberty. Certified seed production will be restricted to USDA HZ 5 and 6 to reduce the potential for genetic shifts within the cultivar. Small quantities of seed for research purposes may be obtained from the USDA-ARS, Lincoln, NE. A technology development and transfer fee will be assessed by the University of Nebraska. Seed of Liberty has been deposited in the National Plant Germplasm System, where it will be available for research purposes. Application will be made for U.S. Plant Variety Protection.

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