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RESEARCH

Soil Quality and Region Influence Performance and Ranking of Switchgrass Genotypes

M. D. Casler,* S. Sosa, A. R. Boe, and S. A. Bonos

ABSTRACT

Development of switchgrass (*Panicum virgatum* L.) as a dedicated bioenergy feedstock requires intensive and extensive breeding programs that include careful and thoughtful consideration of appropriate target populations of environments (TPEs). The purpose of this study was to evaluate region (climate), soil quality, and N fertilization level as potential factors influencing the choice of TPE. A total of 45 switchgrass genotypes were evaluated in uniform field studies at six field sites defined as prime or marginal soils in New Jersey, South Dakota, and Wisconsin. Region and soil quality had strong interactions with genotype, but N fertilization had little impact on genetic variation or ranking of genotypes. Lowland genotypes were considerably more sensitive than upland genotypes to interactions with environmental factors, probably due to these field sites being outside of the traditional lowland adaptation zones. Genotype rankings were highly inconsistent across regions and soil types, indicating that breeding programs that target marginal soils should be located on soils that represent the appropriate TPE. Furthermore, interactions across the three regions suggest that breeding programs for the lowland ecotype should be subdivided into different sets of TPEs, which are largely a function of hardiness zone and annual precipitation. Lastly, even with negligible interactions involving N fertilization level, future definitions of TPEs should be based on minimal or no N fertilizer applications to allow breeders to select plants with greater N-use efficiency, N-scavenging ability, and N-recycling efficiency.

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Abbreviations: GE, genotype × environment; HYE, high-yield environment; LYE, low-yield environment; SS1, Type I sum of squares; TPE, target population of environments.

THE CHOICE of test environments is a critical component of a breeding program. Proper test environments should have the following characteristics: (i) are representative of real-world production conditions (i.e., a high genetic correlation between selection and production environments), (ii) maximize genetic variation and heritability to the greatest extent possible, (iii) are proximal to the central breeding site, including accessibility for personnel and equipment, and (iv) are of sufficient size and scope that land is not a limiting factor. The first two factors can be expressed numerically with classical quantitative genetic statistics useful in making predictions regarding genetic gain, optimizing scarce resources, and choosing the best test environments (e.g., Allen et al., 1978; Rosielle and Hamblin, 1981).

The choice of test environments is relevant to switchgrass (*Panicum virgatum* L.), due to its status as the herbaceous model species for biomass feedstock development by the USDOE (McLaughlin and Kzsos, 2005; Sanderson et al., 2006). At the time of writing this, there are 12 switchgrass breeding programs in North America, and many more genetics or genomics programs that support these breeding efforts. Genotype × environment (GE) interaction is so important in switchgrass that no cultivars

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are broadly adapted across the entire adaptive region and, indeed, few cultivars are broadly adaptive across more than three hardiness zones or two ecoregions (Casler, 2012; Casler et al., 2012). Two cultivars, Alamo and Cave-in-Rock, are exceptions to this "rule," but they are very rare exceptions.

Photoperiod and temperature are the most important factors driving GE interactions in switchgrass (Casler et al., 2004, 2007). Photoperiod drives the evolution of flowering time, whereas temperature drives the evolution of cold and heat tolerance. Southern populations of switchgrass are later in flowering, more heat tolerant, and less cold tolerant than northern populations (Casler, 2012; Casler et al., 2012). Although these three traits have evolved more or less in unison with each other during the Holocene epoch, they are not a priori linked or necessarily pleiotropic to each other. Recent breeding efforts have shown that genetic variation for cold tolerance exists within late-flowering southern populations and the frequency of cold-tolerant plants can be increased by selection within random-mating, late-flowering populations (Casler et al., 2018).

Precipitation or soil moisture capacity is the secondary driver of GE interactions for switchgrass (Berdahl et al., 2005; Casler et al., 2007, 2017). Cultivars bred or evolved in the eastern United States have reduced vigor and/or survivorship in the western United States due to drought; cultivars bred or evolved in the west have reduced vigor and/or survivorship in the east due to lack of disease resistance under humid conditions (Casler, 2012; Casler et al., 2012, 2017). Taken together, these factors have led to the creation of 12 North American breeding programs, each focused on a relatively narrow geographic region, defined partly by photoperiod, temperature, precipitation, and soil moisture (Casler et al., 2015).

Development of a biomass industry that is supported by the diversity that perennial crops add to the landscape will require those crops to be grown under low-input conditions on marginal lands (Robertson et al., 2017). In this context, marginal lands are those that fail to meet local minimum thresholds for economic production of food or feed crops (Shortall, 2013; Richards et al., 2014). For switchgrass, this could include dryland production on nonirrigated pivot corners (Uden et al., 2013), reclaimed surface mines (Brown et al., 2016), buffer strips surrounding sensitive surface waters (Hernandez-Santana et al., 2013; Porter et al., 2015), and highly erosive soils (Hassan et al., 2017). Low-input production conditions will also require the elimination or reduction of N fertilizer, the most expensive of all input factors (Perrin et al., 2008). Ranking of switchgrass cultivars is generally consistent under high-N vs. low-N conditions, but breeding under high-N conditions does not allow breeders to identify potential N-thrifty or N-scavenging genotypes, or those genotypes that are capable of forming beneficial relationships with N-fixing soil microbes (Casler et al., 2017).

The purpose of this study was to clarify GE interaction structure for a highly diverse set of switchgrass genotypes that represent both upland and lowland ecotypes. Specifically, this study focused on addressing the relative importance of region, soil quality, and N fertilization as potential drivers of GE interactions in switchgrass, attempting to answer the question of exactly how best to conduct switchgrass breeding for target populations of environments (TPEs) that are characterized by low-input marginal soils.

MATERIALS AND METHODS

Forty-five genotypes were selected for this experiment, 15 contributed from each of the participating organizations: Rutgers University (New Jersey), South Dakota State University, and the USDA-ARS (Madison, WI). The 45 genotypes represented the following cultivars and populations, with number of genotypes in parentheses: upland populations Carthage (1), Cave-in-Rock (6), Dacotah (14), Shelter (1), Sunburst (1), WS4U (5), and WS8U (5); and lowland populations High Tide (5), Kanlow (3), SL93 (1), and Timber (3). Among the 45 genotypes, 12 represented the lowland ecotype and 33 represented the upland ecotype. The upland populations Carthage and Shelter and the lowland populations High Tide and Timber are of eastern origin, whereas all others originated in the Great Plains, the tallgrass prairie ecosystem (Casler et al., 2015).

Each genotype was collected from a field nursery in early spring 2009 and split into at least 40 clonal ramets for distribution to all collaborators. Clonal ramets were traded between New Jersey, South Dakota, and Wisconsin so that each collaborator had sufficient material to establish equivalent common garden experiments. Four experiments were created within each region by transplanting genotypes in spring or early summer 2009, creating a total of 12 field experiments. The four experiments consisted of the following conditions: prime soil without applied N, prime soil with 100 kg N ha^{-1}, marginal soil without applied N, and marginal soil with 100 kg N ha^{-1}. Prime and marginal soils were achieved by choosing two field sites within each region, for a total of six field sites, belonging to three regions, as defined in Table 1. Paired field sites within each region (marginal vs. prime) were monitored for rainfall and temperature throughout the duration of the study, and no substantial differences were observed within pairs of field sites, so we concluded that the principal factors governing differences within pairs of field sites were the soil characteristics shown in Table 1. Experiments with or without applied N were adjacent to each other within each of the six field sites shown in Table 1.

Each experiment was established as a randomized complete block with three replicates. Plants were spaced 0.9 m apart in all directions, and each experimental unit consisted of a single plant, with approximately three to six tillers at the time of transplanting. Weeds were controlled by a combination of preemergence herbicides and hand weeding as described by Casler (2005). Transplants were irrigated only as needed for establishment, during the first few weeks after transplanting. Plots were clipped and biomass removed after killing frost in autumn 2009.

Table 1. Characteristics of the six field sites and soil types used to evaluate switchgrass genotypes.

Field site	Soil category	Prime vs. marginal defining characteristic	Soil series and taxonomy	Latitude	Longitude	Precip.†	HZ‡
				° N	° W		(°C)
Adelphia, NJ	Prime	High P (400–700 ppm), 27-cm A horizon	Freehold sandy loam soil (fine-loamy, mixed, active, mesic Typic Hapludults)	40.23	74.25	1185	7a (–16.4)
Somerset, NJ	Marginal	Low P (30–60 ppm), 20-cm A horizon	Klinesville loam (loamy-skeletal, mixed, active mesic Lithic Dystrudepts)	40.47	74.53	1180	7a (–16.4)
Aurora, SD	Prime	Well drained and deep silty clay	Brandt silty clay (fine-silty, mixed, mesic, superactive, frigid Calcic Hapludolls)	44.30	96.67	580	4b (–30.0)
Pierre, SD	Marginal	Depth to bedrock ~80 cm, heavy clay	Opal clay (fine, smectitic, mesic Leptic Haplusterts)	44.36	100.00	505	4b (–30.0)
Arlington, WI	Prime	Well drained, 204 mm water storage at 1-m depth§	Plano silt loam (fine-silty, mixed, superactive mesic Typic Argiudolls)	43.30	89.35	850	5a (–27.5)
Hancock, WI	Marginal	Excessively well drained, 71 mm water storage at 1 m§	Plainfield loamy sand (mixed, mesic Typic Udipsamments)	44.11	89.55	825	4b (–30.0)

† Precip., mean annual precipitation, 1996–2015.

‡ HZ, USDA hardiness zone (https://planthardiness.ars.usda.gov/PHZMWeb/), with mean annual extreme minimum temperature shown in parentheses.

§ Obtained from the University of California-Davis SoilWeb (https://casoilresource.lawr.ucdavis.edu/gmap/).

Nitrogen fertilizer was applied in spring 2010 and 2011 to the experiments so designated. Preemergence herbicides and hand weeding were used to keep all experiments weed free. Each plant was hand harvested in autumn 2010 and 2011 near the time of killing frost. Plants were clipped to a stubble height of 10 cm and weighed in the field. A sample of tillers of 100 to 300 g was collected, weighed, and dried at 60°C for 7 d to determine dry matter concentration, which was used to adjust plant biomass to a dry-matter basis.

Data were analyzed by mixed-models analysis (Littel et al., 1996) with year, region, soil quality, N fertilizer, and genotype as fixed effects and block as the only random effect. Contrasts were used to estimate and test the effect of ecotype. Analyses were performed separately for each region and combined across field sites. Residuals were normally distributed but showed evidence of heterogeneity associated with region, soil quality, and N fertilization. As such, residuals were modeled as individual "variance groups" using the repeated measures function within Proc Mixed (Littel et al., 1996). Year was treated as a repeated measure and modeled using compound symmetry covariance structure with heterogeneous variances between years. All fixed effects involving genotypes were evaluated on the basis of both P value and percentage contribution to the Type I sum of squares (SS1). Statistical significance was based on $P < 0.05$, and biological significance was based on SS1 >5%.

Linear regressions and phenotypic correlation coefficients were computed between the 24 arrays of mean plant biomass (2 yr × 3 regions × 2 soil types × 2 N rates) to help explain the interactions of genotype with year, region, soil type, and N rate. Phenotypic correlation coefficients that were homogeneous and represented similar GE interaction structures were pooled (Steel et al., 1997). Additionally, a mixed-models analysis was conducted separately for each of the 24 combinations of region, soil quality, N level, and ecotype. The F values for genotypes from these 24 analyses were then subjected to a mixed models analysis to identify associations with region, soil quality, N level, or ecotype.

Finally, phenotypic plasticity was computed for each of the 45 genotypes by first computing the GE matrix of means (12 environments = 3 regions × 2 soil types × 2 N rates), then computing the phenotypic SD for each genotype across the 12 environments. Reaction norms are typically used to evaluate phenotypic plasticity, but they do not easily lend themselves to an overall evaluation of plasticity across 12 environments. Hence the phenotypic SD was used as a measure of environmental sensitivity (Falconer, 1990).

RESULTS

Genotype was the most important source of variation, the largest of the fixed effects, excluding the main effects of the environmental factors (Table 2). Ten of the 15 interaction terms involving genotype were significant ($P < 0.01$), but only three of these were biologically significant: the interactions of genotype with region, soil type, and region × soil type. These three interactions plus the main effect of genotype accounted for 79% of the sum of squares for the fixed effects listed in Table 2. Although there was some significance to interactions involving year and N rate, these were never >2.7% of the sum of squares and, combined, accounted for only 13 (all year terms) or 12% (all N terms) of the sum of squares.

For upland genotypes, there was considerable concordance across all six field sites, including both regions and soil types (Table 3, above the diagonal). All of these correlations were significant ($P < 0.05$) and positive. For comparative purposes, the pooled correlation between years was 0.75 ± 0.06 and the pooled correlation between N rates was 0.73 ± 0.10. This is in contrast with the lowland genotypes, which were highly inconsistent across regions and soil types (Table 3, below the diagonal). Three field sites had similar ranking and relative responses for the lowland genotypes: both South Dakota sites and Wisconsin prime. The other three sites largely gave completely different results, uncorrelated with each other or with the concordant groups that included the South Dakota sites.

These interactions resulted in a strong disagreement in ranking of genotypes across the six sites (Table 4).

Table 2. Analysis of variance results associated with the fixed effects of switchgrass genotype and all genotype × environment interactions involving year (Y), region (R), soil type (S), and N fertilization rate (N) for plant biomass measured in 2010 and 2011.

Fixed effect	df	*F* value	*P* value	%SS1†
				%
Genotype	44	34.45	<0.01	31.4
Genotype × R	88	11.74	<0.01	21.4
Genotype × Y	44	1.90	<0.01	1.7
Genotype × R × Y	88	1.24	0.07	2.3
Genotype × S	44	11.56	<0.01	10.5
Genotype × S × R	81	9.23	<0.01	15.5
Genotype × S × Y	44	2.96	<0.01	2.7
Genotype × S × R × Y	79	1.41	0.01	2.3
Genotype × N	44	1.67	<0.01	1.5
Genotype × N × R	84	1.53	<0.01	2.7
Genotype × N × Y	44	1.02	0.44	0.9
Genotype × N × R × Y	82	0.99	0.51	1.7
Genotype × N × S	44	1.69	<0.01	1.5
Genotype × N × S × R	78	1.39	0.02	2.2
Genotype × N × S × Y	44	0.70	0.93	0.6
Genotype × N × S × R × Y	71	0.64	0.99	0.9

† %SS1, percentage of the Type I sums of squares for all genotype and genotype × environment interaction effects.

Genotypes ranked within the top four were dominated by lowland genotypes (8 of 13), which was largely as expected. Remarkably, 7 of the 11 germplasm sources contributed at least one genotype to those in Table 4, the genotypes that ranked within the top four at one or more of the six sites. Only Dacotah, WS4U, Shelter, and Sunburst were not represented (Shelter and Sunburst likely because they each contributed only one genotype to the experiment; Dacotah and WS4U likely due to their relatively low productivity, as observed in previous studies: Casler and Boe, 2003; Casler, 2010). Lowland genotypes of eastern origin (Timber and High Tide) were ranked high at both New Jersey sites and the South Dakota prime soil. Lowland genotypes of western origin (Kanlow and SL93) ranked high within all three regions, essentially at five of the six sites, excluding only the Wisconsin marginal soil. The five upland genotypes shown in Table 4 were included only as a result of high rankings at either South Dakota soil or the Wisconsin marginal soil.

There were no differences among genotypes from Timber, High Tide, or the western lowland populations at the two most extreme field sites: New Jersey prime soil and Wisconsin marginal soil (Table 5). At the other four sites, High Tide was always lower in plant biomass, on

Table 3. Phenotypic correlation coefficients among regions and soil types for plant biomass of switchgrass genotypes (upland genotypes above the diagonal, *n* = 33; lowland genotypes below the diagonal, *n* = 12).

Region and soil type	New Jersey prime	New Jersey marginal	South Dakota prime	South Dakota marginal	Wisconsin prime	Wisconsin marginal
New Jersey prime		0.75**	0.68**	0.86**	0.50**	0.44**
New Jersey marginal	0.25		0.50**	0.61**	0.63**	0.59**
South Dakota prime	−0.12	0.58		0.92**	0.39*	0.62**
South Dakota marginal	−0.27	0.45	0.81**		0.53**	0.56**
Wisconsin prime	−0.13	0.42	0.86**	0.59*		0.73**
Wisconsin marginal	−0.48	−0.39	0.01	0.10	0.08	

* Significantly different from zero at the 0.05 probability level.

** Significantly different from zero at the 0.01 probability level.

Table 4. Rankings of 13 switchgrass genotypes (out of 45 total) for mean plant biomass, averaged over years, replicates, and N rates for each of the six locations shown in Table 1. The criterion for inclusion in this table was to be ranked within the top four within at least one of the six locations (those rankings are shown in bold and italicized font).

Genotype no.	Origin†	Ecotype	New Jersey marginal soil	New Jersey prime soil	South Dakota marginal soil	South Dakota prime soil	Wisconsin marginal soil	Wisconsin prime soil
101	Timber	Lowland	***1***	***3***	8	40‡	45	44
110	Timber	Lowland	11	15	***1***	40‡	27	37
115	Timber	Lowland	10	***1***	7	8	32	40
114	High Tide	Lowland	17	***4***	30	21	44	34
102	Kanlow	Lowland	***2***	***2***	6	***4***	39	***3***
103	Kanlow	Lowland	***3***	14	***3***	***1***	33	***1***
104	Kanlow	Lowland	6	7	***2***	***2***	42	***2***
113	SL93	Lowland	***4***	13	5	14	16	***4***
111	Carthage	Upland	5	10	9	5	***4***	13
112	Cave-in-Rock	Upland	21	8	***4***	***3***	11	15
311	WS8U	Upland	7	45‡	13	12	***2***	11
312	WS8U	Upland	20	28	17	6	***3***	9
315	WS8U	Upland	19	17	10	9	***1***	6

† Timber and High Tide originated in the eastern United States; Kanlow and SL93 originated in the Great Plains of the central United States.

‡ Genotype was ranked last or tied for last due to mortality during the first or second year of the experiment.

average, than the western lowland populations. Timber was lower in plant biomass than the western lowland populations only at two of the western field sites: South Dakota prime soil and Wisconsin prime soil.

Separate ANOVA results for the three regions are shown in Table 6, illustrating the dramatic difference across these three regions. For New Jersey, there were no significant effects for lowland genotypes—all variability was between upland and lowland ecotypes or within the upland ecotype. The genotype × soil type interaction accounted for 32% of the sum of squares (Table 6), illustrated in Fig. 1A. The slope of the linear regression for upland genotypes was significantly greater than one (Table 7), indicating they expressed their genetic differences in productivity much more on the New Jersey prime soil than the New Jersey marginal soil. Likewise, lowland genotypes were 3.8× more productive than upland genotypes on the prime soil (2387 vs. 620 g plant^{-1}), but only 2.5× more productive on the marginal soil (409 vs. 162 g plant^{-1}). Furthermore, the response to N fertilizer, as indicated by the slope of the regressions for plant biomass with vs. without fertilizer (Fig. 1B and 1C), was 2.5× greater for the prime soil than the marginal soil (Table 6, 1.21 vs. 0.46).

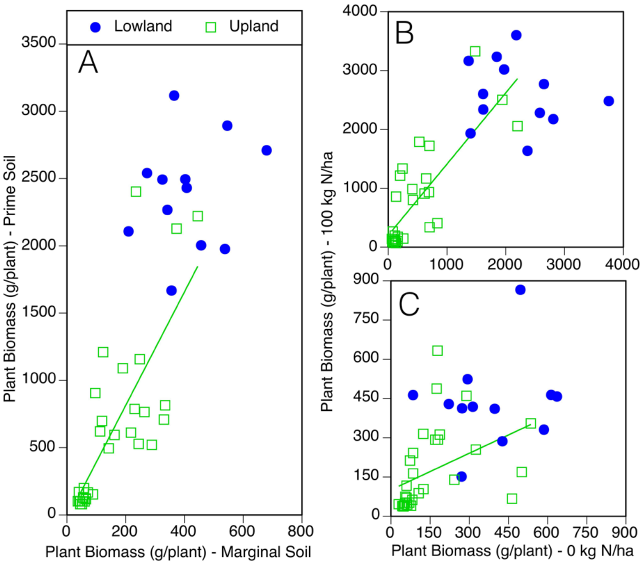

Fig. 1. Scatterplots and linear regressions of plant biomass for lowland and upland ecotypes of switchgrass in New Jersey: (A) prime soil vs. marginal soil, (B) 100 vs. 0 kg N ha^{-1} on the prime soil, and (C) 100 vs. 0 kg N ha–^{1} on the marginal soil. Statistics of these regressions are shown in Table 6. Note that the *x* and *y* axes of Panel A are not on the same scale.

Table 5. Mean plant biomass, averaged over years, replicates, and N rates, for eastern vs. western lowland switchgrass genotypes evaluated at six locations.

Location (region and soil quality)	Timber genotypes	High Tide genotypes	Western lowland genotypes	LSD(0.01)
		g plant^{-1}		
New Jersey marginal soil	467	351	487	122
New Jersey prime soil	2494	2376	2322	NS†
South Dakota marginal soil	748	216	759	169
South Dakota prime soil	519	296	754	180
Wisconsin marginal soil	102	106	120	NS
Wisconsin prime soil	184	184	1249	228

† NS, nonsignificant.

Table 6. Analysis of variance results associated with the fixed effects of soil type, N rate, genotypes, and interactions for switchgrass plant biomass evaluated in three regions of the United States (New Jersey, South Dakota, and Wisconsin).

		New Jersey			South Dakota			Wisconsin		
Fixed effect	df	*F* value	*P* value	SS1%†	*F* value	*P* value	SS1%†	*F* value	*P* value	SS1%†
Soil type (S)	1	572.67	<0.01		0.42	0.54		129.49	<0.01	
Nitrogen (N)	1	14.32	0.01		0.58	0.47		4.56	0.10	
S × N	1	10.38	0.01		1.96	0.20		2.28	0.21	
Ecotype (Eco)	1	337.30	<0.01	38.0	335.41	<0.01	31.5	0.14	0.71	0.0
Lowland genotype (Low)	11	1.00	0.46	2.6	11.84	<0.01	26.9	102.92	<0.01	33.3
Upland genotype (Up)	32	16.17	<0.01	17.5	18.36	<0.01	34.0	12.12	<0.01	18.2
Eco × S	1	173.75	<0.01	19.6	0.92	0.34	0.1	34.64	<0.01	1.5
Low × S	11	0.78	0.66	2.0	0.90	0.54	1.7	100.71	<0.01	32.6
Up × S	32	9.56	<0.01	10.0	1.21	0.24	1.7	4.93	<0.01	7.4
Eco × N	1	0.53	0.47	0.1	0.87	0.35	0.1	7.39	0.01	0.3
Low × N	11	1.25	0.27	3.2	0.34	0.97	0.8	2.08	0.04	0.7
Up × N	32	2.02	<0.01	2.1	0.63	0.92	0.9	2.33	<0.01	3.5
Eco × S × N	1	0.25	0.62	0.0	1.21	0.27	0.1	1.46	0.23	0.1
Low × S × N	11	1.29	0.25	3.3	1.49	0.21	1.8	2.59	0.01	0.8
Up × S × N	32	1.72	0.03	1.5	0.26	1.00	0.4	1.08	0.37	1.6

† SS1%, percentage of the Type I sums of squares for all genotype and genotype × environment interaction effects.

Table 7. Linear regression statistics characterizing various genotype × environment interactions for switchgrass plant biomass of 12 lowland genotypes and 33 upland genotypes: New Jersey shown in Fig. 1, South Dakota shown in Fig. 2, and Wisconsin shown in Fig. 3.

Region and ecotype	*y* axis†	*x* axis†	Intercept			Slope				R^2
			Estimate	SE	*P* value	Estimate	SE	*P* ($b = 0$)	*P* ($b = 1$)	
			g plant^{-1}	g plant^{-1}						
New Jersey										
Lowland	Prime	Marginal	2112	419	<0.01	0.67	0.98	0.51	0.75	0.05
Upland	Prime	Marginal	–36	139	0.80	4.29	0.71	<0.01	<0.01	0.59
Lowland	P100	P0	2910	588	<0.01	–0.14	0.26	0.59	<0.01	0.03
Upland	P100	P0	191	137	0.17	1.21	0.19	<0.01	0.26	0.63
Lowland	M100	M0	370	127	0.02	0.16	0.30	0.61	0.02	0.03
Upland	M100	M0	87	35	0.02	0.46	0.17	0.01	<0.01	0.23
South Dakota										
Lowland	Prime	Marginal	42	119	0.74	1.08	0.21	<0.01	0.71	0.84
Upland	Prime	Marginal	36	25	0.16	1.18	0.10	<0.01	0.08	0.86
Lowland	P100	P0	254	128	0.10	0.55	0.18	0.03	0.04	0.64
Upland	P100	P0	28	22	0.21	0.74	0.06	<0.01	<0.01	0.87
Lowland	M100	M0	–67	114	0.59	1.30	0.23	<0.01	0.22	0.87
Upland	M100	M0	24	20	0.24	0.88	0.08	<0.01	0.12	0.85
Wisconsin										
Lowland	Prime	Marginal	404	545	0.48	1.23	4.73	0.80	0.96	0.01
Upland	Prime	Marginal	70	70	0.32	2.33	0.39	<0.01	<0.01	0.54
Lowland	P100	P0	–118	56	0.06	1.21	0.08	<0.01	0.02	0.96
Upland	P100	P0	50	71	0.48	0.95	0.14	<0.01	0.73	0.58
Lowland	M100	M0	73	44	0.13	0.65	0.47	0.20	0.48	0.16
Upland	M100	M0	174	52	<0.01	0.59	0.46	0.21	0.37	0.05

† M0, marginal soil, 0 kg N ha^{-1}; M100, marginal soil, 100 kg N ha^{-1}; P0, prime soil, 0 kg N ha^{-1}; P100, prime soil, 100 kg N ha^{-1}.

Conversely, for South Dakota, there were no interactions involving either soil type or N rate, with the main effect of genotypes accounting for 92% of the sum of squares for genotypes and GE interactions (Table 6). This result is reinforced by the high phenotypic correlations between the two South Dakota sites for both upland and lowland genotypes (Table 3). The lack of interactions for South Dakota is illustrated in Fig. 2, which shows the general agreement in both ranking and expression of genotype performance for prime vs. marginal soils (Fig. 2A) and between the two N rates (Fig. 2B and 2C). Furthermore, all regression slopes in Fig. 2 were remarkably similar, all significantly different from zero ($P < 0.05$) with only two deviating significantly from unity (Table 7). All but one of these linear regressions had $R^2 > 0.8$.

Lastly, the Wisconsin region displayed yet another unique genotype × environment structure (Table 5), with a strong genotype main effect (51% of the sum of squares) and a strong genotype × soil type component (42% of the sum of squares). Most of the genotype × soil type interaction was due to the lowland genotypes, which were unable to express their genetic differences at the Wisconsin marginal site (Fig. 3A). Surprisingly, all 12 lowland genotypes survived at the Wisconsin marginal site, but most were severely stunted with low vigor. Upland genotypes exhibited a small amount of genotype × soil type interaction in Wisconsin, partly due to changes in ranking ($R^2 = 0.54$) and partly due to differential expression of genetic differences ($b > 1$, $P < 0.01$, Fig. 3A, Table 6). Upland and lowland genotypes both expressed their genetic differences at the Wisconsin prime site (Fig. 3B), with a strong positive relationship between plant biomass under the two N rates. Conversely, due to the compression of genetic variability at the Wisconsin marginal site, there was no relationship in performance or agreement in ranking of genotypes between the two N rates for this site (Fig. 3C).

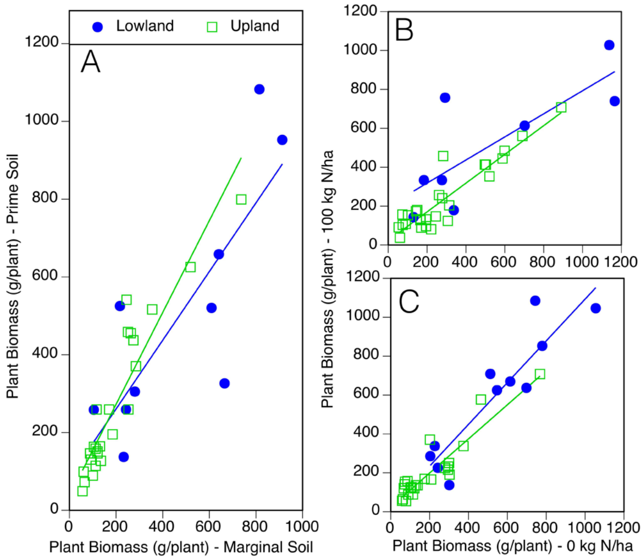

Fig. 2. Scatterplots and linear regressions of plant biomass for lowland and upland ecotypes of switchgrass in South Dakota: (A) prime soil vs. marginal soil, (B) 100 vs. 0 kg N ha–^{1} on the prime soil, and (C) 100 vs. 0 kg N ha^{-1} on the marginal soil. Statistics of these regressions are shown in Table 6. Note that the *x* and *y* axes of Panel A are not on the same scale.

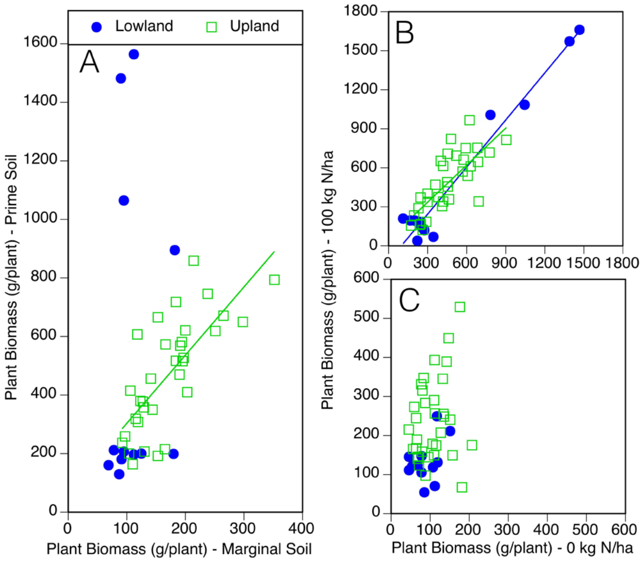

Fig. 3. Scatterplots and linear regressions of plant biomass for lowland and upland ecotypes of switchgrass in Wisconsin: (A) prime soil vs. marginal soil, B) 100 vs. 0 kg N ha^{-1} on the prime soil, and (C) 100 vs. 0 kg N ha^{-1} on the marginal soil. Statistics of these regressions are shown in Table 6. Note that the *x* and *y* axes of Panel A are not on the same scale.

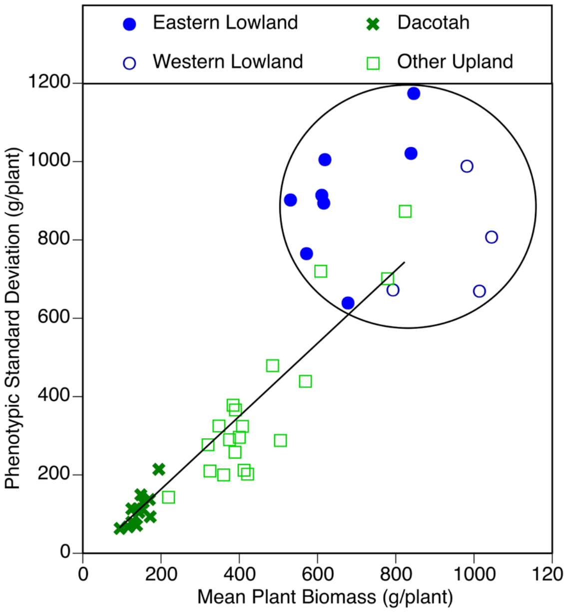

Fig. 4. Scatterplot between mean plant biomass and phenotypic SD across environments as a measure of phenotypic plasticity. Linear regression for all upland genotypes: $Y = -53.6 + 0.97X$ ($R^2 = 0.87$, $P < 0.01$).

Soil quality was the only factor that influenced the size of the *F* value for genotypes; region, N level, and ecotype were all nonsignificant. Mean *F* values were 8.16 ± 2.47 for prime soils and 2.98 ± 0.81 for marginal soils ($P = 0.04$). Treating genotypes within ecotypes to be a random effect, these mean values translated into broad sense heritability estimates of 0.63 vs. 0.42 for prime and marginal soils, respectively.

Across all 45 genotypes, phenotypic plasticity was strongly correlated with mean performance for plant biomass (Fig. 4). This relationship was due largely to variation within upland genotypes and between upland and lowland genotypes. The relationship was linear within upland genotypes, and lowland genotypes were clearly separated from all but three upland genotypes. Dacotah had the lowest mean plant biomass and its genotypes demonstrated little plasticity across the wide range of environments in this experiment. Genotypes from the other upland cultivars were highly variable in plasticity, but their plasticity was highly predictable from mean performance. The lack of a relationship between mean plant biomass and plasticity for plant biomass within lowland genotypes indicates that some other factors were responsible for the observed variation in phenotypic plasticity.

DISCUSSION

Genotype × Environment Interaction

This study provides additional evidence that there is a strong GE interaction component for switchgrass productivity on an east–west gradient. A number of earlier studies of switchgrass cultivars have pointed to the importance of this interaction (Casler and Boe, 2003; Casler et al., 2007, Casler et al., 2017), but this is the first study to show that this interaction is also manifested on an individual-genotype level. Furthermore, this interaction is present within both upland and lowland ecotypes, indicating that this interaction must be one of the drivers in making decisions about test environments, breeding objectives, germplasm pools, and target regions for cultivar deployment. If switchgrass is going to become a major biomass crop for conversion to bioenergy, the breeding programs in these three regions will all be critical for development of regionally optimal cultivars for biomass production.

The six field sites in this experiment resulted in five different patterns of genotypic performance and ranking, with the two South Dakota sites essentially mimicking each other, due to the absence of genotype × soil type interaction. Considering the two South Dakota sites as one, this leaves five distinct "ecozones," which are defined by differences in soil quality, annual precipitation, and minimum annual temperature (Table 1). South Dakota and Wisconsin were similar in hardiness zone (minimum annual temperature), but contrasting in annual precipitation, whereas New Jersey was highest in both metrics, essentially the most favorable environment for switchgrass production, especially for lowland genotypes (Fig. 1–3).

The performance of genotypes in New Jersey and Wisconsin strongly suggests that breeding new cultivars for use on marginal soils should be conducted on marginal soils that are chosen to reflect production environments. The South Dakota results are likely an anomaly, due to the Pierre site not being sufficiently "marginal" to result in differential responses compared with the Brookings site. Similar means and responses to N fertilizer between Brookings and Pierre suggest that neither site could be considered marginal for the purposes of this study. Defining and choosing sites that can clearly and reliably be considered to be marginal can be difficult in agronomic research, as evidenced in a previous study with seven pairs of prime vs. marginal sites, only one of which resulted in a clear and obvious difference in mean productivity and ranking of cultivars (Casler et al., 2017). One of the obvious difficulties of conducting this type of study is to identify sufficiently "marginal" sites that are available for research. Each region has literally thousands of hectares of marginal lands, probably based on a number of different criteria or metrics (Richards et al., 2014), but this type of experimental research cannot be conducted on many of these sites, due to distance and logistical issues.

Two lines of evidence in this study point to reduced tolerance for lowland genotypes to reduced moisture availability. First, the tendency of the western lowland genotypes from Kanlow and SL93 to have greater plant biomass at the western field sites (Table 5) suggests the possibility of preferential adaptation to this lower rainfall region. This was consistent for High Tide, and partially consistent for Timber (observed at two of the four western field sites). Second, the extremely poor performance of most lowland genotypes at Hancock, WI, the marginal site, defined by a sandy soil with low water-holding capacity, suggest that most lowland genotypes were unadapted to this site, not due to weather, but due to the soil characteristics. Furthermore, the four lowland genotypes that ranked high at the Wisconsin prime site (Fig. 3) originated from Kanlow and SL93, both of western (Great Plains) origin. Similarly, in South Dakota, Kanlow genotypes tended to have the highest rankings on both soil types. This is tangible evidence that lowland populations show differential adaptation across a broad east–west transect that extends from the Great Plains to the Atlantic Seaboard and is dominated largely by a precipitation gradient. Furthermore, this is largely a subtle response, not an overwhelming response that could be used to classify germplasm as "adapated" vs. "unadapted." High rankings of some Timber genotypes in South Dakota and of Kanlow genotypes in New Jersey indicate that this is a subtle difference in adaptation, in which adaptation varies across this gradient by degrees, not leaps and bounds. Conversely, this is simply not an issue for upland genotypes, which do not display any evidence of differential adaptation across this east–west transect or to different soil types.

Adaptational variation associated with mean annual precipitation has been well documented within the tallgrass prairie ecosystem. Increased precipitation leads to plants with increased aboveground plant biomass, but not necessarily to increased root biomass (Epstein et al., 1997; Zhou et al., 2009). Soil moisture availability is regulated by both precipitation and soil characteristics, both of which drive plant functional composition of tallgrass prairies (Paruelo et al., 1999; Knapp et al., 2001, 2015). Grasses with the C_4 photosynthetic pathway tend to be fairly drought tolerant as a rule and this trait likely explains their dominance at the western edge of this cross-continental precipitation gradient (Lane et al., 1998; Knapp et al., 2001). As mean annual precipitation increases, drought sensitivity of grasses that have evolved under those conditions also increases (Knapp et al., 2015). Similar to observations from this study, big bluestem has demonstrated subtle genotypic differences in adaptive responses to a precipitation gradient that ranges from 470 to 1100 mm (Rouse et al., 2011; Johnson et al., 2015). Genotypes from dryland environments have greater survivorship under dryland conditions and less diversity in functional disease resistance gene homologs, presumably because there is reduced disease pressure under dryland conditions (Rouse et al., 2011; Johnson et al., 2015). Much like the current study on switchgrass (Tables 4 and 5), adaptive responses for big bluestem were subtle, not overwhelming and not observed for all genotypes or for all evaluation sites.

Nitrogen: To Fertilize or Not

As a general observation, N fertilization had little effect on genotype rankings and expression of genetic variability in this study. The only exceptions were on the marginal soils of New Jersey and Wisconsin. In Wisconsin, this lack of relationship appears to have been caused exclusively by the compression of genetic variability on the marginal soil, essentially preventing genotypes from expressing their production potential. The general rule seems to be that N fertilization does not strongly influence cultivar rankings. A previous study of upland and lowland cultivars, including 14 sites with both prime and marginal soils, showed a pooled phenotypic correlation for biomass yield between 0 and 100 kg N ha^{-1} of $r = 0.64 \pm 0.08$ (Casler et al., 2017).

This phenomenon is very common for grain yield of annual crops, which generally displays a high positive correlation across different N rates in wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), and maize (*Zea mays* L.) (Bänziger et al., 1997; Barraclough et al., 2010; Hitz et al., 2017; Li et al., 2017; Russell et al., 2017). The switchgrass breeding and selection experiments of Rose et al. (2007) are the only notable exceptions, in which a relatively low genetic correlation was observed between high-yield environments (HYEs) and low-yield environments (LYEs). In this study, the difference between LYEs and HYEs included numerous confounded factors (depth of A and B horizons, ~100 vs. ~200 cm; 0 vs. 90 kg N ha^{-1}; zero vs. recommended rates of P and K fertilizer; and none vs. supplemental irrigation), so that the low correlation between LYEs and HYEs could not be attributed to the difference in N fertilization. Rose et al. (2007) observed that selection for increased plant biomass was more effective in LYEs than in HYEs, regardless of which environments were used for evaluation of selected populations.

Although the high correlations observed in this study (Table 3) and that of Casler et al. (2017) suggest that similar genotypes will be selected under N-fertilized vs. unfertilized conditions, there are some risks associated with continued fertilization of breeding nurseries and evaluation plots.

First, N is expensive, both as a production input (Perrin et al., 2008) and as it impacts the environment (Robertson et al., 2017). Nitrogen fertilization of switchgrass results in NO_3 leaching into groundwater and N_2O emissions into the atmosphere (Erisman et al., 2010; Robertson et al., 2017).

Second, switchgrass does not always respond to N fertilizer with increased biomass production (Wullschleger et al., 2010; Robertson et al., 2017). Fertilization with N leads to increased concentration of N in the biomass, which may be removed if harvests are made before much of this N can be recycled (Lemus et al., 2008; Jung and Lal, 2011). Just-in-time harvesting is going to be a major component of cellulosic biomass production systems, creating the situation where agronomic or ecological optimal growth stages for harvesting are unrealistic and biomass harvest occurs over perhaps 3 to 4 mo in the autumn and early winter (Shinners et al., 2017). Optimal rates of N fertilization have been determined as the maximum amount to which switchgrass responds in a linear manner but does not result in significant leaching into groundwater ($\sim$100 kg N ha^{-1} yr^{-1}; Vogel et al., 2002). Improvements to N-use efficiency of switchgrass would allow a reduction in this rate, the frequency of application, or both.

Third, genetic variability exists for the rate and timing by which switchgrass recycles N (Porter, 1966; Yang et al., 2009) suggesting the possibility for genetic improvement. The most effective way for breeders to accomplish this goal may be to conduct selection under conditions where N is either limiting or just barely adequate for biomass production—this is a hypothesis that should be tested. This approach could help to ensure that roots and rhizomes act as an efficient sink for aboveground N (Clark, 1977; Schwartz and Amasino, 2013; de Vries and Bardgett, 2016).

Fourth, rhizobacteria or endophytic bacteria can have significant growth-promotion impacts, such as biological N fixation, nutrient acquisition, and increased biomass production (Farrar et al., 2014; Tkacz and Poole, 2015). Arbuscular mycorrhizal fungi can acquire nutrients from organic and inorganic sources in the soil and transfer some of these nutrients to host plants (Hodge and Storer, 2015). Nitrogen fertilization of switchgrass reduces the ability of the soil microbiome to evolve into a diverse community that resembles that under long-term prairies (Oates et al., 2016). Host–microbe associations are heritable (Peiffer et al., 2013), and this variation can be used to identify and select genotypes with superior abilities to benefit from these associations from an agronomic viewpoint (Lima et al., 1987; Urquiaga et al., 1992; Dong et al., 2018). There is some evidence for genetic or ecotypic variability in host–microbe nutrient dynamics within switchgrass, supporting a hypothesis that high-biomass cultivars will be better able to support a bacterial community with high nitrogenase activity compared with low-biomass cultivars (Rodrigues et al., 2017).

In contrast, there are almost no risks associated with elimination of N fertilizer on breeding nurseries and evaluation plots. There are no theoretical or practical reasons to expect that breeding gains will be greater or more efficient under high-N vs. low-N conditions (Rose et al., 2007; Casler et al., 2017).

Implications for Switchgrass Breeding

The anonymous breeder's axiom "you get what you select for" should be one of the driving principles in the choice of selection and evaluation environments to breed biomass-type switchgrass cultivars. Breeding late-flowering lowland switchgrass for adaptation to northern environments (e.g., Hardiness Zones 3–5) provides a relevant example. Gains have been made by selection within Hardiness Zone 5, specifically near Madison, WI, and Lincoln, NE, but these gains have not led to increased persistence or biomass yield in Hardiness Zones 3 or 4, due to insufficient selection pressure under these more extreme conditions (Casler et al., 2018). It is clear that continued improvement in cold or freezing tolerance for Hardiness Zones 3 and 4 will not occur until selection nurseries are routinely planted in representative environments.

Plant breeders have traditionally focused selection efforts on HYEs, opting to create the most favorable environment for genetic expression in the naïve viewpoint that this practice favors the greatest gains (Bänziger and Cooper, 2001). More recently, many breeding programs are shifting objectives to align with lower-input production systems or even organic systems, incorporating principles of participatory research (Dawson et al., 2008; Ceccarelli and Grando, 2009). Practical results from maize and wheat breeding programs have demonstrated that indirect selection for low-N environments conducted under high-N conditions is considerably less effective than direct selection under low-N conditions (Bänziger et al., 1997; Machado and Fernandes, 2001; Presterl et al., 2003; Brancourt-Hulmel et al., 2005). Theoretical considerations support these results, that the requirements for high genetic correlation and increased heritability under high-N conditions vs. low-N conditions are simply too great to make indirect selection efficient (Allen et al., 1978; Rosielle and Hamblin, 1981; Atlin and Frey, 1989).

Similarly, sward-plot evaluations of switchgrass cultivars have shown that there can be significant changes in ranking across different soil types and N rates, depending on soil conditions (Casler et al., 2017).

These principles hold regardless of the source of the GE interaction, whether the environmental component is caused by a difference in N fertilizer, soil quality, soil moisture, or temperature. Furthermore, they are generally true across all crops, including grain crops, fodder crops, and biomass crops. Indeed, Brummer and Casler (2014) indicated that a wide range of indirect selection efforts to improve forage or biomass yield of perennial crops have failed to produce gains equivalent to those achieved by direct selection. Toward this end, switchgrass breeding programs should be focused on subdivisions of the overall region to which the species is adapted. The choice of TPE for breeding should be defined according to hardiness zone, soil moisture availability, and, to the extent possible, soil quality. The results of this study support the existing proposal of eight "breeding zones," defined largely by hardiness zone and annual precipitation (Casler, 2012; Casler et al., 2012, 2015). Atlin et al. (2000) demonstrated the effectiveness of this form of subdivision but included the caveat that each subdivision should retain a sufficient number of test sites to adequately evaluate economic traits across the entire subdivision. Uniform testing collaborations are an effective mechanism to accomplish this for switchgrass (e.g., Casler et al., 2017, 2018).

Lastly, there is little justification for continued selection of switchgrass on prime farmland where fertility tends to be high and food or fodder crops are commonly grown. Logistics may be considerably more difficult for breeders to find long-term reliable marginal sites that are conveniently located, but the risks associated with breeding on prime farmland are simply too great to continue with the status quo. Because lands can be "marginal" for one or more of many reasons (Richards et al., 2014), switchgrass breeders might do well to identify and define appropriate selection sites as LYEs, as was done by Rose et al. (2007), where soil fertility, soil depth, and moisture availability were all factors in creating low-yield conditions. Regardless of the underlying definition of the LYEs, their results were in agreement with quantitative genetic theory—increases in plant biomass in the LYEs were greatest when selection was conducted directly in the LYEs.

Conflict of Interest

The authors declare that there is no conflict of interest.

Acknowledgments

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