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## Genotypic variability in mineral composition of switchgrass

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### ABSTRACT

Switchgrass (*Panicum virgatum* L.) is a warm season perennial grass with great potential as an energy crop in the USA. It is widely adapted to many regions of the country, produces large amounts of biomass, serves as a useful forage grass, and provides ecosystem services that benefit soil and water quality and wildlife. Biological and thermochemical technologies are being developed to convert herbaceous biomass, including switchgrass, to energy. The objective of this research was to determine the effect of genotype and production environment on the concentration of minerals that affect the suitability of switchgrass for thermochemical conversion and to quantify the amount of potassium (K) and phosphorus (P) removed from the production system by harvest of the aboveground biomass, a measure of the sustainability of the practice. Straw dry biomass contained from 1.3 to 6.4 kg Mg<sup>-1</sup> and from 6.2 to 15.8 kg Mg<sup>-1</sup> of P and K, respectively. Variability in aluminum (Al), calcium (Ca), chloride (Cl), K, P, silicon (Si), and sulfur (S) concentrations across locations was relatively high, ranging from twofold (Al) to eightfold (Cl). Location had a strong impact on mineral concentrations among switchgrass genotypes evaluated in this study. Latitude of origin impacted the Cl and Si concentrations measured in plant tissues, but none of the other minerals analyzed in this study. Upland and lowland cytotypes explained some of the observed differences, but population × location interactions were the primary source of variability in the concentration of these minerals.

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### 1. Introduction

Switchgrass is a warm season grass that is native to the USA and Canada and highly productive in the prairies of North America (Parrish and Fike, 2005). It serves as important forage because of its capacity to grow during the hot-summer months when lack of rainfall limits the growth of most other grasses (Moser and Vogel, 1995). Stands of switchgrass also provide a variety of ecosystem services which impact wildlife diversity and grassland bird species (Paine et al., 1996; Henningson and Best, 2005; Roth et al., 2005; Lusk et al., 2006). With the exception of several western states, the geographical range of switchgrass covers most of the USA (Casler et al., 2004). Upland and lowland cytotypes which tend to have different genetic and agronomic traits (Sanderson et al., 1996; Casler et al., 2004) have been described (Hultquist et al., 1996). This wide geographical adaptation, combined with high yield and flexibility to use switchgrass as livestock forage make this crop attractive for utilization as feedstock for bioenergy production (Perlack et al., 2005).

The US Department of Energy has identified switchgrass as the most likely bioenergy crop from which liquid fuels could be

produced to reduce the dependency on imported fossil fuel (Wright, 1994; McLaughlin and Kszos, 2005). During the past 15 years, switchgrass has been evaluated for potential as a bioenergy crop in the midwestern USA (Vogel et al., 2002; Casler and Boe, 2003), the southern Great Plains (Sanderson et al., 1996, 1999), and Canada (Madakadze et al., 1998).

General approaches to convert switchgrass to liquid fuels include fermentation and thermochemical technologies (McKendry, 2002), although both require considerable refinement to become economical. Of the two approaches, thermochemical conversion of straw to syngas, a mixture of carbon monoxide, hydrogen, and other gases, and subsequently to biopower or liquid fuels, has great potential for small to mid scale energy production biorefineries where transportation costs limit economic conversion at a large scale (Boateng et al., 2007). Thermochemical conversion in a gasification reactor involves heating the feedstock under restricted oxygen conditions to produce syngas which is subsequently converted to an energy product (McKendry, 2002). Previous research demonstrated that the quality and yield of syngas produced from switchgrass was temperature-dependent and that the greatest carbon (C) conversion efficiency occurred at temperatures in excess of 750 °C (Boateng et al., 2006). At operational temperatures where C conversion efficiency is high, Si, which is abundant in grass feedstocks, and S react with Al, Cl, K, and other alkalis to form sticky

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glass-like silicates and oxides referred to as slag, along with corrosive alkali sulfates (Miles et al., 1996; Jenkins et al., 1998; Thompson et al., 2003). Slag formation and corrosive alkalis increase maintenance cost, reduce the lifespan and utility of gasifier hardware and diminish the economic feasibility of converting these feedstocks to fuel (Miles et al., 1996). Reduced mineral content at harvest improves the combustion characteristics of biomass (Nordin, 1994).

Genetic improvement of switchgrass may reduce the potential for slag and corrosive alkali formation during thermochemical processing of switchgrass. While there has been considerable development of switchgrass germplasm adapted to specific production environments, little is known about the genetic variability in mineral concentration in this grass. In this study, the concentrations of Al, Ca, Cl, K, P, Si, and S, and in aboveground biomass of selected upland and lowland switchgrass cytotypes grown at various locations over 2 years were quantified. We included analyses of Al because previous work (Rengel and Robinson, 1989) demonstrated that Al impacts uptake of other macronutrients measured in this study. Our objective was to determine the effect of genotype on the concentration of minerals that affect the suitability of switchgrass for thermochemical conversion and to quantify the amount of K and P removed from the production system by harvest of the aboveground biomass.

## 2. Methods

### 2.1. Sites and cultivar descriptions

Four upland populations of switchgrass including cultivars Cave-in-Rock, Trailblazer, Sunburst, and Blackwell, along with two lowland cytotypes, Kanlow and NL94-1, were harvested from five locations in Spooner, WI (Spn; 45°49'N, 91°54'W), Arlington, WI (Arl; 43°20'N, 89°23'W); Mead, NE (Mea; 41°13'N, 96°29'W); Manhattan, KS (Man; 39°25'N, 96°35'W); and Stillwater, OK (Stl; 36°7'N, 96°5'W). Soil types included an Omega loamy sand (sandy, mixed, frigid Typic Haplorthod) at Spooner, Plano silt loam (fine-silty, mixed, superactive, mesic Typic Argiudoll) at Arlington, Sharpsburg silt loam (fine, smectitic, mesic Typic Argiudoll) at Mead. Haynie very fine sandy loam (coarse-silty, mixed, superactive, calcareous, mesic Mollic Udifluent) at Manhattan, and a Kirkland silt loam (fine, mixed, superactive, thermic Udertic Paleustoll) at Stillwater. The experimental design, location details, growing, harvesting, and plant tissue preparation procedures were previously described (Casler et al., 2004). In brief, the experimental design at each location was a randomized complete block with three replicates at Spooner, four at Manhattan, five at Arlington and Stillwater, and six at Mead. Weeds were controlled by clipping and herbicide application as described (Casler et al., 2004). Plots were fertilized in spring with 112 kg N ha<sup>-1</sup>. A flail harvester was used to harvest a 0.9 m swath from the center of each plot in late summer after most populations had completed anthesis. Samples representing the 1999 crop year were available from all locations except Manhattan. All locations were represented in the 2000 crop year.

### 2.2. Mineral extraction and analysis

Plant dry matter was ground through a 2-mm screen of a Wiley-type mill (Thomas Scientific, Swedesboro, NJ, USA) and a 1-mm screen of a cyclone mill. Minerals were extracted from plant samples utilizing microwave-assisted acid digestion (EPA method 3052) with an Ethos D microwave station (Milestone, Monroe, CT) and analyzed for Al, Ca, K, P, S, and Si by inductive coupled plasma-optical emission spectroscopy (ICP-OES) (Isaac and Johnson, 1985; Perkin-Elmer Life and Analytical Sciences, Shelton, CT). A

quality control check standard (QCCS), representing the approximate midpoint of the calibration range for each mineral was included as the first sample in each set and was repeated following every tenth sample. All QCCS value determinations were within 10% of the actual concentration. Plant tissue samples (25 g) were extracted with 100 ml of deionized water and shaken for 30 min at 350 rpm and then filtered through Whatman Qualitative No. 42 filters (Florham Park, NJ) that had been washed three times with 1% H<sub>2</sub>SO<sub>4</sub> (v/v) and deionized water. The filtrate was analyzed colorimetrically for Cl (QuickChem method 10-117-07-1-C) on a Lachat flow injection autoanalyzer (Hach Co., Loveland, CO).

### 2.3. Statistical analyses

Data were initially subjected to analysis of variance (PROC GLM, SAS, Statistical Analysis System Institute, Cary, NC) to identify interactions among locations, years, cultivars, and cytotypes. Since mineral concentrations in these populations were not different ( $P=0.05$ ) between 1999 and 2000, data from the 2 years were pooled for subsequent analyses and data were tabulated to show interactions between cultivar, location, and cytotype. Plant tissues were not available from each location both years so means represent data where  $n=10$  for Arl;  $n=8$  for Man;  $n=8$  for Stl;  $n=12$  for Mea;  $n=6$  for Spn. Statistical analyses of mean differences among populations, locations, and comparisons between upland and lowland cytotypes were calculated by analysis of variance, where locations and years were considered random and varieties and cytotypes were considered fixed. Main and interaction effects were considered significant at  $P \leq 0.05$ , unless otherwise stated. Means of all minerals were evaluated for main effects and interaction of the locations, latitude of origin, years, and varieties were compared utilizing Tukey' studentized range (HSD) test (SAS).

## 3. Results

Mean monthly temperature and precipitation data from May to September showed that Stl and Spn received approximately average rainfall in 1999 and 2000 while Arl and Mea received less than average precipitation during one of these years (Table 1). Location and cultivar accounted for much of the variability observed and location  $\times$  cultivar interactions had an effect for five of the seven minerals (Table 2). There were location interactions with the concentrations of all seven minerals. Latitude of origin affected the concentrations of Cl and S, but not that of the other five minerals. There was no apparent relationship between temperature and rainfall with mineral concentration (Tables 3–9). Genotypic variability in Al, Ca, Cl, K, P, S, and Si concentrations was affected by the location at which the crop was grown and in some cases, the variability was associated with cytotype.

Differences in Al concentrations within a location were relatively small among these populations and generally lower in the two northernmost locations, Arl and Spn (Table 3). Interactions of Al concentration with cytotype and Man and Mea, the two locations at intermediate latitudes used in this study.

Chloride concentrations within these genotypes varied nearly eightfold, ranging from 513 to 4258 mg kg<sup>-1</sup> (Table 4). On average, plants grown at Man and Mea contained less Cl whereas those harvested at Stl contained the greatest concentrations. Interactions between Cl concentration and cytotype occurred at Arl, Mea, and Stl. At Arl, and Mea, Cl concentrations were greatest in upland cytotypes, in contrast to Stl where lowland cytotypes contained more Cl.

The concentrations of K in these populations were generally lower in plants grown at Man (Table 5). Lowland cytotypes contained more K in plants grown at Mea, Spn, and Stl. The Kanlow

**Table 1**

Mean temperature and precipitation data for five locations at which switchgrass populations were grown.

Location/ month	1999 Precipitation (mm)	2000 Precipitation (mm)	1999 Temperature (°C)	2000 Temperature (°C)
<i>Arlington WI</i>				
May	10.0	26.6	16	16
June	13.4	18.2	20	18
July	8.7	8.7	24	21
August	6.4	8.4	19	22
September	3.6	7.9	15	17
Total	42.1	69.8		
<i>Mead NE</i>				
May	15.6	5.8	16	18
June	12.9	14.3	21	21
July	7.2	9.7	26	23
August	9.2	3.0	22	25
September	8.1	2.0	17	19
Total	53.0	34.8		
<i>Manhattan KS</i>				
May	13.1	6.5	18	19
June	19.1	14.7	22	23
July	4.6	5.5	28	27
August	6.3	1.8	25	30
September	9.7	2.4	19	23
Total	52.8	30.8		
<i>Spooner WI</i>				
May	11.5	11.3	16	15
June	11.1	12.2	19	17
July	18.1	19.0	23	21
August	13.8	12.2	20	20
September	9.4	1.7	15	15
Total	64.0	56.4		
<i>Stillwater OK</i>				
May	11.5	16.7	20	21
June	20.7	19.3	24	24
July	4.9	13.1	28	27
August	2.5	0.1	29	30
September	15.5	0.1	21	24
Total	10.0	49.3		

plant population grown at Spn contained the greatest concentrations of K among these genotypes.

The concentration of P was generally lower in plants grown at Arl, where NL94-1 contained the lowest concentration recorded in our study (Table 6). Interactions between P concentration and cytotype occurred, but were location dependent. At Arl and Man, upland cytotypes contained more P. In contrast, lowland cytotypes contained greater concentrations of P at Spn. Latitude of origin had little effect on the P measured in these populations.

The concentrations of Si ranged from 3818 to 13956 mg kg<sup>-1</sup> and were strongly dependent on location (Table 7). Populations grown at Arl and Spn contained the lowest concentrations, approximately half that measured in the other locations. Interactions

**Table 2**

P-values for partial analysis of variance of mineral concentrations in plant tissues from six switchgrass populations derived from two latitudes of origin (North and South) grown at Arlington, WI, Spooner, WI, Manhattan, KS; Mead, NE; and Stillwater, OK.

Sources of variation	df	Al	Ca	Cl	K	P	Si	S
Latitude of origin	1	0.2081	0.1171	0.0073	0.6158	0.5663	0.0002	0.3301
Location	4	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Cultivar	5	0.7300	0.1396	<0.0001	0.0002	<0.0001	<0.0001	<0.0001
Location × cultivar	20	0.3958	0.0107	<0.0001	0.0016	0.2992	<0.0001	0.0124
Location × latitude of origin	4	0.9138	0.1241	0.0025	0.2446	0.0154	0.0035	0.7745

**Table 3**

Aluminum (Al) concentration (mg kg<sup>-1</sup> dry plant tissue) in aboveground biomass of six populations of switchgrass grown in 1999 and 2000 at Arlington (Arl), and Spooner (Spn), WI; Manhattan (Man), KS; Mead (Mea), NE; and Stillwater (Stl), OK. Interactions of Al concentration with location, cultivar, and cytotype based on initial analysis of variance to test for significant interactions. Cytotype interactions represent pooled cultivar data including four upland and two lowland cytotypes. Data are expressed as mean mg kg<sup>-1</sup> dry plant tissue (n = 10 for Arl; n = 8 for Man; n = 8 for Stl; n = 12 for Mea; n = 6 for Spn).

Cultivar (cytotype–latitude of origin) <sup>a</sup>	Arl	Man	Mea	Spn	Stl
Cave-in-Rock (U–S)	77	54	97	47	93
Blackwell (U–S)	50	94	104	55	106
Sunburst (U–N)	46	66	111	47	96
Trailblazer (U–N)	53	89	94	44	97
Kanlow (L–N)	57	63	102	65	91
NL94-1 (L–N)	61	50	89	44	98
Pooled LSD (P = 0.05)	23	38	21	34	24
Cytotype					
Lowland	59	57	92	55	94
Upland	57	76	103	48	98
P-values	0.31	0.00	0.00	0.26	0.14

<sup>a</sup> U, upland; L, lowland; S, southern, south of 40° N; N, northern, north of 40° N for upland, north of 34° N for lowland.

**Table 4**

Chloride (Cl) concentration (mg kg<sup>-1</sup> dry plant tissue) in aboveground biomass of six populations of switchgrass grown in 1999 and 2000 at Arlington (Arl), and Spooner (Spn), WI; Manhattan (Man), KS; Mead (Mea), NE; and Stillwater (Stl), OK. Interactions of Cl concentration with location, cultivar, and cytotype based on initial analysis of variance to test for significant interactions. Cytotype interactions represent pooled cultivar data including four upland and two lowland cytotypes. Data are expressed as mean mg kg<sup>-1</sup> dry plant tissue (n = 10 for Arl; n = 8 for Man; n = 8 for Stl; n = 12 for Mea; n = 6 for Spn).

Cultivar (cytotype–latitude of origin) <sup>a</sup>	Arl	Man	Mea	Spn	Stl
Cave-in-Rock (U–S)	1605	828	661	1345	3682
Blackwell (U–S)	1168	716	650	1406	3632
Sunburst (U–N)	1136	762	513	1391	2781
Trailblazer (U–N)	1026	836	588	1444	3606
Kanlow (L–N)	1216	653	522	1503	4088
NL94-1 (L–N)	1002	830	560	1333	4258
Pooled LSD (P = 0.05)	205	340	187	298	211
Cytotype					
Lowland	1109	742	541	1418	4173
Upland	1234	786	603	1396	3425
P-values	0.00	0.46	0.00	0.67	0.00

<sup>a</sup> U, upland; L, lowland; S, southern, south of 40° N; N, northern, North of 40° N for upland, North of 34° N for lowland.

between Si concentration and cytotype occurred at all five locations, and in four of the five, upland cytotypes contained greater concentrations of Si. Kanlow and Blackwell plant populations generally contained more Si whereas NL94-1 often contained the least. The impact of cytotype was greater than that of latitude of origin.

Plants grown at Man contained lower S concentrations than those produced at the other locations (Table 8). Interactions of S concentration with cytotype occurred at four of the five locations

**Table 5**

Potassium (K) concentration (mg kg<sup>-1</sup> dry plant tissue) in aboveground biomass of six populations of switchgrass grown in 1999 and 2000 at Arlington (Arl), and Spooner (Spn), WI; Manhattan (Man), KS; Mead (Mea), NE; and Stillwater (Stl), OK. Interactions of K concentration with location, cultivar, and cytotype based on initial analysis of variance to test for significant interactions. Cytotype interactions represent pooled cultivar data including four upland and two lowland cytotypes. Data are expressed as mean mg kg<sup>-1</sup> dry plant tissue ( $n = 10$  for Arl;  $n = 8$  for Man;  $n = 8$  for Stl;  $n = 12$  for Mea;  $n = 6$  for Spn).

Cultivar (cytotype–latitude of origin) <sup>a</sup>	Arl	Man	Mea	Spn	Stl
Cave-in-Rock (U–S)	10,679	7750	10,140	9086	8087
Blackwell (U–S)	9538	7765	9936	10,389	8986
Sunburst (U–N)	9619	7792	8457	8210	6481
Trailblazer (U–N)	9741	6192	9691	10,074	7674
Kanlow (L–N)	11,304	7674	11,316	15,806	8368
NL94-1 (L–N)	9006	7969	11,175	10,211	8609
Pooled LSD ( $P = 0.05$ )	1643	2724	1450	2389	1690
Cytotype					
Lowland	10,155	7822	11,246	13,008	8489
Upland	9894	7375	9556	9440	7807
$P$ -values	0.13	0.35	0.00	0.00	0.00

<sup>a</sup> U, upland; L, lowland; S, southern, south of 40° N; N, northern, north of 40° N for upland, north of 34° N for lowland.

**Table 6**

Phosphorus (P) concentration (mg kg<sup>-1</sup> dry plant tissue) in aboveground biomass of six populations of switchgrass grown in 1999 and 2000 at Arlington (Arl), and Spooner (Spn), WI; Manhattan (Man), KS; Mead (Mea), NE; and Stillwater (Stl), OK. Interactions of P concentration with location, cultivar, and cytotype based on initial analysis of variance to test for significant interactions. Cytotype interactions represent pooled cultivar data including four upland and two lowland cytotypes. Data are expressed as mean mg kg<sup>-1</sup> dry plant tissue ( $n = 10$  for Arl;  $n = 8$  for Man;  $n = 8$  for Stl;  $n = 12$  for Mea;  $n = 6$  for Spn).

Cultivar (cytotype–latitude of origin) <sup>a</sup>	Arl	Man	Mea	Spn	Stl
Cave-in-Rock (U–S)	1890	5656	4319	3177	2844
Blackwell (U–S)	1702	5418	4951	3633	2604
Sunburst (U–N)	1889	5389	4404	2229	2925
Trailblazer (U–N)	2369	6445	4364	4030	3671
Kanlow (L–N)	2210	4504	4392	5075	3041
NL94-1 (L–N)	1297	3876	4548	4179	2912
Pooled LSD (0.05)	658	1092	601	958	677
Cytotype					
Lowland	1754	4190	4470	4627	2977
Upland	1963	5727	4509	3267	3011
$P$ -values	0.00	0.00	0.49	0.00	0.63

<sup>a</sup> U, upland; L, lowland; S, southern, south of 40° N; N, northern, north of 40° N for upland, north of 34° N for lowland.

where upland cytotypes contained the greater concentrations. Latitude of origin did not appear to impact the concentration of this mineral. NL94-1 populations contained the least concentration of S at four of five locations.

Location differences in Ca concentrations were small relative to those observed for the other minerals (Table 9). Calcium concentrations ranged from 2290 (NL94-1 at Man) to 5296 (Kanlow at Spn) mg kg<sup>-1</sup>. Populations of upland cytotypes contained more Ca at three locations, but latitude of origin had little effect on Ca.

#### 4. Discussion

The mineral concentrations of herbaceous biomass have great impact on the suitability of switchgrass and other perennial grasses as feedstock for thermochemical conversion processes (Jenkins et al., 1998; Boateng et al., 2006). Mineral constituents including K, Cl, S, and Si, react to form slag deposits and corrosive compounds at temperatures in the range of 700 °C (Baxter, 1993; Jenkins et al., 1998). Operation of thermal reactors at reduced

**Table 7**

Silicon (Si) concentration (mg kg<sup>-1</sup> dry plant tissue) in aboveground biomass of six populations of switchgrass grown in 1999 and 2000 at Arlington (Arl), and Spooner (Spn), WI; Manhattan (Man), KS; Mead (Mea), NE; and Stillwater (Stl), OK. Interactions of Si concentration with location, cultivar, and cytotype based on initial analysis of variance to test for significant interactions. Cytotype interactions represent pooled cultivar data including four upland and two lowland cytotypes. Data are expressed as mean mg kg<sup>-1</sup> dry plant tissue ( $n = 10$  for Arl;  $n = 8$  for Man;  $n = 8$  for Stl;  $n = 12$  for Mea;  $n = 6$  for Spn).

Cultivar (cytotype–latitude of origin) <sup>a</sup>	Arl	Man	Mea	Spn	Stl
Cave-in-Rock (U–S)	6955	11219	10296	4908	9736
Blackwell (U–S)	8636	13956	10501	5516	10909
Sunburst (U–N)	7640	12526	11310	4535	12969
Trailblazer (U–N)	8724	12543	9574	3818	12441
Kanlow (L–N)	6958	13033	8369	6529	8945
NL94-1 (L–N)	7197	8860	7497	4533	8146
Pooled LSD (0.05)	1383	2294	1263	1756	1242
Cytotype					
Lowland	7077	10947	7933	5531	8546
Upland	7989	12561	10420	4694	11514
$P$ -values	0.00	0.00	0.00	0.00	0.00

<sup>a</sup> U, upland; L, lowland; S, southern, south of 40° N; N, northern, north of 40° N for upland, north of 34° N for lowland.

**Table 8**

Sulfur (S) concentration (mg kg<sup>-1</sup> dry plant tissue) in aboveground biomass of six populations of switchgrass grown in 1999 and 2000 at Arlington (Arl), and Spooner (Spn), WI; Manhattan (Man), KS; Mead (Mea), NE; and Stillwater (Stl), OK. Interactions of S concentration with location, cultivar, and cytotype based on initial analysis of variance to test for significant interactions. Cytotype interactions represent pooled cultivar data including four upland and two lowland cytotypes. Data are expressed as mean mg kg<sup>-1</sup> dry plant tissue ( $n = 10$  for Arl;  $n = 8$  for Man;  $n = 8$  for Stl;  $n = 12$  for Mea;  $n = 6$  for Spn).

Cultivar (cytotype–latitude of origin) <sup>a</sup>	Arl	Man	Mea	Spn	Stl
Cave-in-Rock (U–S)	983	567	950	900	701
Blackwell (U–S)	949	675	1002	1012	768
Sunburst (U–N)	1023	750	1039	877	790
Trailblazer (U–N)	1031	633	1133	1027	775
Kanlow (L–N)	1037	567	890	1108	725
NL94-1 (L–N)	886	467	917	781	690
Pooled LSD (0.05)	90	148	82	131	92
Cytotype					
Lowland	961	517	903	944	707
Upland	996	656	1031	954	758
$P$ -values	0.00	0.00	0.00	0.66	0.00

<sup>a</sup> U, upland; L, lowland; S, southern, south of 40° N; N, northern, north of 40° N for upland, north of 34° N for lowland.

temperatures frequently produces syngas with reduced heating value. Boateng et al. (2006) showed that the heating value of non-condensable gases produced during pyrolysis of switchgrass peaked around 900 °C. Genetic selection to reduce mineral accumulation by switchgrass has potential to reduce the risk of slag formation at optimal reactor operation temperatures, but a genetic approach is dependent upon the existence of variability in mineral concentration. Among the minerals quantified in our study, the least variability was observed in Ca, K, and S, three macronutrients that are important components in slag formation.

Variability in the minerals analyzed in this study was dependent upon complex location × cultivar interactions which made prediction of mineral concentrations within switchgrass populations at a given location difficult. While differences in the concentration of specific minerals between populations were measured at each of the five locations, the relative ranking of the populations was not consistent across all locations. Casler and Boe (2003) reported that interactions among harvest date, growing site, year

**Table 9**

Calcium (Ca) concentration ( $\text{mg kg}^{-1}$  dry plant tissue) in aboveground biomass of six populations of switchgrass grown in 1999 and 2000 at Arlington (Arl), and Spooner (Spn), WI; Manhattan (Man), KS; Mead (Mea), NE; and Stillwater (Stl), OK. Interactions of Ca concentration with location, cultivar, and cytotype based on initial analysis of variance to test for significant interactions. Cytotype interactions represent pooled cultivar data including four upland and two lowland cytotypes. Data are expressed as mean  $\text{mg kg}^{-1}$  dry plant tissue ( $n = 10$  for Arl;  $n = 8$  for Man;  $n = 8$  for Stl;  $n = 12$  for Mea;  $n = 6$  for Spn).

Cultivar (cytotype–latitude of origin) <sup>a</sup>	Arl	Man	Mea	Spn	Stl
Cave-in-Rock (U–S)	3396	3372	3475	4485	3133
Blackwell (U–S)	3164	3816	3617	4721	3641
Sunburst (U–N)	2965	3348	3783	4170	3879
Trailblazer (U–N)	3481	3570	3475	4017	4017
Kanlow (L–N)	3150	2695	3147	5296	3270
NL94-1 (L–N)	3460	2290	3124	4042	3059
Pooled LSD (0.05)	475	787	433	690	488
Cytotype					
Lowland	3305	2493	3135	4669	3165
Upland	3251	3527	3588	4481	3668
P-values	0.28	0.00	0.00	0.10	0.00

<sup>a</sup> U, upland; L, lowland; S, southern, south of 40° N; N, northern, north of 40° N for upland, north of 34° N for lowland.

and cultivar also made biomass yield unpredictable among six upland cultivars of switchgrass. The impact of location on Cl illustrates the variability associated with location. Plant Cl concentration was generally lowest in populations grown at Mead, NE where Sunburst contained approximately  $500 \text{ mg kg}^{-1}$ . In contrast, Sunburst populations grown at other locations were not always the lowest in Cl concentration. At Stillwater, OK, Sunburst plants contained the least Cl, but the concentrations detected were fivefold greater than those measured in plants grown at Mead, NE.

The Si concentrations were of special interest because this mineral is critical in slag formation. Similar to other minerals, complex location  $\times$  cultivar interactions influenced the concentration of Si measured in plant tissues. The lowest concentrations of Si measured in this study were in populations grown at Spooner, WI. The relatively low Si concentrations measured in those populations were unexpected because the soil at Spooner is sandy (rich in Si) relative to the other sites. Accessibility of Si by plants is largely dependent upon the rate of water uptake (availability), and weathering of the Si parent molecule which frees Si into soil solution. Minerals such as quartz (e.g., quartz sand) are highly resistant to weathering processes, making Si less available to plants (Mengel and Kirby, 1987). Location effects did not appear to be associated with temperature or precipitation encountered during most of the growing season. Studies of Si accumulation during heat adaptation suggest that warmer temperatures are accompanied by an increased formation of silica bodies and increased silica-containing leaf pubescence in grasses (Banowetz et al., 2008). In the present study, the least Si occurred in plants grown at the two coolest locations (Spooner and Arlington, WI) while the greatest silica concentrations were observed in plants grown at Manhattan and Stillwater, KS, the two locations with warm mid-summer temperatures.

The basis for the complex location effect remains unclear. Hopkins et al. (1995a,b) reported genotype  $\times$  environment effects on forage yield, quality, holocellulose, and lignin concentrations among diverse switchgrass populations propagated at three Midwest locations over two different cropping seasons. Despite the complexity of these interactions, they were able to identify populations that consistently ranked high in yield and quality. Casler and Boe (2003) also demonstrated genotype  $\times$  environment interactions in six switchgrass populations planted at two locations where Cave-in Rock consistently ranked lowest in ash content, a favorable trait for thermochemical conversion processes. Lewan-

dowski et al. (2003) found similar location effects in a study of 15 genotypes of *Miscanthus*, a  $C_4$  perennial grass, planted at five European locations. They showed that delayed harvest reduced biomass yields, but improved combustion qualities by significantly reducing ash, Cl, K, N, and moisture content. Delayed harvest also reduced yields of reed canarygrass (Christian et al., 2006) and switchgrass (Christian et al., 2002; Vogel et al., 2002). Management practices like chemical weed control, residue incorporation, soil drainage improvement, or soil fertilization, which impact soil characteristics including pH, also affect mineral uptake and subsequently their concentrations in plant tissues (Parrish and Fike, 2005).

One factor that might have contributed to variability in our study was plant physiological stage. Populations of all cultivars used in this study were harvested at the same time at each of the five locations and consequently, the plant samples represented, to some extent, differential maturity stages among genotypes. Dien et al. (2006) demonstrated that differences in physiological maturity correlated with mineral concentrations in three potential bio-energy crops, including switchgrass. Physiological maturity of Cave-in-Rock population impacted condensable gas yield in switchgrass pyrolysis products at temperatures  $<750^\circ\text{C}$  (Boateng et al., 2006).

The range of mineral concentrations in these switchgrass populations was similar to that reported for Cave-in-Rock in a previous study (Dien et al., 2006), although the concentrations of silica measured in our study were on the lower end of the range they reported. They indicated that the possibility of soil contamination may have contributed to elevated Si concentrations in their plant populations. Quantities of P and K removed from the production system by harvest of the biomass ranged from  $1.3$  to  $6.4 \text{ kg Mg}^{-1}$  (P) and from  $6.2$  to  $15.8 \text{ kg Mg}^{-1}$  (K), depending on the location and cultivar. The concentrations of Cl, K, and P measured in our study were comparable to those reported by Christian et al. (2002), who evaluated seven varieties of switchgrass that included Cave-in-Rock, Sunburst, and Kanlow, three of the six plant population evaluated in our study. These independent reports utilizing different analytical approaches confirmed the general range and baseline of mineral concentrations that should be anticipated in genetic approaches to improve the suitability of switchgrass for thermochemical conversion technologies. Ultimate and proximate analyses of three switchgrass populations which included Cave-in-Rock and Kanlow showed few genotypic differences in Cl concentration and that of mineral oxides, although Cave-in-Rock had somewhat greater ash (Lemus et al., 2002). The variability observed by Lemus et al. (2002) among these components between harvest years was in general agreement with our findings.

## 5. Conclusion

Genotypic variability in the concentration of these minerals occurred within these populations, but location effects made prediction of mineral concentrations within a given population difficult. Ranking of populations across the locations for all the minerals showed that NL94-1 and Sunburst most frequently contained the lowest concentration of the respective mineral. In contrast, Cave-in-Rock, Blackwell, and Kanlow frequently contained the greatest concentrations of minerals. Sunburst accumulated relatively low concentrations of Si in cooler locations, but contained greater concentrations of this mineral at Stillwater, OK, the warmest location. In general, silica concentrations were greater at locations with warm-summer temperature.

The contributions of cytotypes and latitude of origin to mineral concentration was at least partially masked by unidentified environmental factors. At Mead, NE, the intermediate latitude in this

study, mineral concentrations in upland northern cultivars were similar to that of upland southern cultivars. The genotypic variability observed among these switchgrass populations suggests that genetic improvement in the suitability of switchgrass for thermochemical conversion technologies is possible, although much remains to be learned about environmental factors that impact mineral concentration.

The strong location effect demonstrated in this study suggests that site-specific management practices or other environmental influences also may have large effect on mineral concentrations in these switchgrass populations. Further research to identify management or other non-genetic effects, as well as genotype is needed to determine the most effective means to lower the concentrations of minerals that impact thermochemical conversion processes.

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