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# Does Selection for Seedling Tiller Number in Perennial Biomass Feedstocks Translate to Yield and Quality Improvements in Mature Swards?

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## Abstract

Breeding for seedling traits in herbaceous perennial biomass feedstocks that translate into increased biomass yield or quality in established swards could accelerate the development of perennial grass cultivars for bioenergy or forage. In previously reported research, breeding for single large tillers (ST) or multiple tillers (MT, ≥3) six weeks after planting for two generations in big bluestem (*Andropogon gerardii* Vitman) and switchgrass (*Panicum virgatum* L.) base populations produced ST and MT populations that differed significantly for seedling and mature plant traits including biomass yield in spaced planted nurseries. Our objective was to evaluate these ST and MT populations in sward trials to determine the effect of these genetic changes on biomass yield and quality when the plants were grown in competitive sward conditions. Big bluestem monocultures of the base, ST, and MT populations were evaluated at three locations in Nebraska in 2001 and 2002 as randomized complete block experiments with four replicates. Switchgrass monocultures of the base, ST, and MT populations were evaluated in 2003, 2004, and 2005 near Mead, NE as a randomized complete block with six replicates. In both big bluestem and switchgrass, the ST and MT populations did not consistently differ from the base population or each other for biomass yield or forage quality. These results demonstrate the importance of evaluating perennial grasses in sward trials and not relying solely on greenhouse-grown plants or space-planted nurseries to develop selection criteria and make selection decisions.

## Keywords

Seedling Tiller Number, Perennial Biomass

## 1. Introduction

Improving perennial grasses like big bluestem and switchgrass for yield, digestibility, or other selected traits requires multiple generations and extensive field evaluation [1]. For example, the releases of "Bonanza" and "Goldmine" big bluestem cultivars required three generations of breeding for increased yield and digestibility, small plot yield trials, seed increase nurseries, followed by grazing trials, a process requiring nearly 20 years from initiation to cultivar release [2] [3] [4]. This improvement through breeding resulted in Bonanza producing 14% more kg of beef $ha^{-1}$ than the Pawnee base population [2]. However, if selecting for traits in the seedling stage results in the expression of the desired trait in established swards, perennial grass cultivar development could be accelerated and would provide an important contribution to perennial grass breeding.

Breeding work to improve seedling vigor in switchgrass and big bluestem was initiated in 1978 by selecting for seedling weight at 4-wk after emergence using a stratified mass selection breeding system. Three breeding generations were conducted. The experimental populations developed by the breeding work were evaluated in both greenhouse and field trials and demonstrated that breeding progress had been made in improving seedling shoot weight [5]. The base populations used in this breeding work were "Pawnee" big bluestem and "Pathfinder" switchgrass. During the selection process, some seedlings were identified that had single large tillers while others had multiple tillers. This led to the work reported previously by Smart *et al.* [6] [7] [8] in which divergent breeding was conducted for single and multiple tiller seedlings in big bluestem and switchgrass populations. The selection work was conducted in a greenhouse, selected plants were intermated in isolated field polycross nurseries, and the resulting new experimental strains were evaluated in both greenhouse and field trials. The base populations, Pawnee C3SW big bluestem and Pathfinder C3SW switchgrass, used in the single and multiple tiller breeding work were the cycle 3 (C3) populations developed by three breeding generations for seedling shoot weight (SW).

Divergent selection for seedling weight and seedling tiller number from Pawnee C3SW big bluestem and Pathfinder C3SW switchgrass developed two unique seedling populations after two breeding generations for each species, one with large single tillers (ST) and one with multiple tillers (MT, ≥3) 6 to 8 wk after planting [6] [7]. When evaluated in the greenhouse, the rankings of the derived populations for both species for tiller number were MT > base population > ST and for seedling shoot weight were ST > base population > MT [6]. In subsequent field evaluation trials, the divergent populations did not differ for seedling shoot weight, seedling root weight, and morphological root stage, but shoot stage values were greater for MT than ST populations [7]. There were no differences between populations within a species for stand establishment for two years in field plots [7]. The authors suggested that ST and MT populations did not differ in field establishment because root systems were not altered by the breeding to improve seedling shoot weight and tiller number. Mature plants of the big bluestem and switchgrass base MT and ST populations were then evaluated in space-transplanted field nurseries [8]. As mature plants in the space-transplanted trials, the ST populations had fewer tillers per plant, greater tiller weight, plant height, and biomass yields than the MT populations when harvested at the end of the growing season [8]. All differences were significant at $P < 0.05$ except for plant biomass yield for the big bluestem populations. These results demonstrated that breeding for seedling tiller number affected plant morphology of mature big bluestem and switchgrass plants when evaluated in space-transplanted nurseries.

The objective of this study was to evaluate big bluestem and switchgrass base, ST (selection for a single large tiller in the seedling stage), and MT (selection for multiple tillers in the seedling stage) populations in sward trials to determine if these differences in plant morphology affected stands, biomass yield and quality. In addition, the developmental morphology as measured by mean stage count (MSC), tiller density, and tiller demographics in big bluestem was also evaluated. We tested the hypothesis that divergent selection for single or multiple tillers in the seedling stage in big bluestem and switchgrass will result in populations that differ in aboveground biomass yield and quality, and tiller population dynamics in established swards. If the hypothesis is supported, we would expect an outcome similar to the previous research done on space-planted stands by Smart *et al.* [6] [7] [8]. If supported, selecting for seedling tiller number may be a viable approach to accelerate big bluestem and switchgrass cultivar development for bioenergy, livestock production, and conservation.

## 2. Materials and Methods

The big bluestem and switchgrass populations described previously that were bred for differences in seedling tiller number and morphology were evaluated in sward trials. The experimental strains and cultivars evaluated for the two species were:

| Big bluestem | Switchgrass |
|---|---|
| Pawnee C3SW (base population) | Pathfinder C3SW (base population) |
| Pawnee ST (single seedling tiller) | Pathfinder ST |
| Pawnee MT (multiple seedling tillers) | Pathfinder MT |
| Pawnee (check parent cultivar) | Trailblazer (check control cultivar) |

These big bluestem and switchgrass strains were evaluated by species in separate sward trials. Because of the breeding methods used in their development, these strains and cultivars are differentiated populations of heterogenous plants.

### 2.1. Big Bluestem

The big bluestem portion of the study was conducted in three environments using three sets of sward yield trials that were seeded in 1999. The trials were located at the University of Nebraska's Eastern Nebraska Research, Education, and Extension Center (ENREEC) near Mead, NE (41˚09'16"N; 96˚25'30"W), the South Central Research and Extension Center near Clay Center, NE (40˚34'25"N; 98˚07'46"W), and the Northeast Research and Extension Center near Concord, NE (42˚23'27"N; 96˚57'01"W). The climate at all locations is continental with most of the annual precipitation occurring in the growing season. Weather data were obtained from permanent weather stations at each location [9]. Two trials were planted at each site. One trial had a July pre-heading harvest followed by a late autumn regrowth harvest while the other set of trials had a single post-heading harvest in August. The two-harvest management system would be used for producing high-quality hay for beef cattle while the single August harvest likely would be used for a biomass harvest for bioenergy.

The strains were planted in May 1999 at Mead, Clay Center, and Concord, NE. Soil on the Mead site was a Sharpsburg silty clay loam (fine, smectitic, mesic Typic Argiudoll), a very deep, moderately well-drained soil formed in loess. Soil on the Clay Center site was a Crete silt loam (fine, smectitic, mesic Pachic Argiustoll), a very deep, moderately well-drained soil formed in loess with 0% to 2% slope. Soil on the Concord site was on Alcester silty clay loam (fine-silty, mixed, mesic, Cumulic Haplustoll) soil, a very deep, well and moderately well-drained soil formed in silty colluvial-alluvial sediments with a 0% to 2% slope. The Mead site is 50 km west of Omaha, Clay Center is 155 km southwest of Mead, and Concord is 135 km north of Mead. Plots (1.5 × 3.1 m) were established at each location by seeding 430 pure live seed $m^{-2}$ in rows spaced 15 cm apart with a no-till plot drill (Hege Inc., Waldenburg, Germany). Each trial at a location was a randomized complete block with four replicates per strain or cultivar. Each site was uniformly cropped with soybean [*Glycine max* (L.)] for at least one year prior to establishment. Broadleaf and grassy weeds were controlled with 293 ml $ha^{-1}$ imazapic [Plateau®; (±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-methyl-3-pyridinecarboxylic acid] plus 1.1 kg a.i. $ha^{-1}$ atrazine [6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine] applied as a pre-emergent immediately after planting. In the spring of each year, the standing dead material was removed either by burning or by harvesting the standing dead material to a 10 cm cutting height. Fertilizer was applied in late May of each year at 110 kg N $ha^{-1}$ as ammonium nitrate.

Big bluestem frequency of occurrence was measured in 2000 using the frequency grid [10]. To determine potential differences among big bluestem populations on tiller demographics and morphology, swards of each population at each location were sampled immediately prior to initiation of reproduction in the two-harvest set of plots in July 2002 from 2, 0.1 $m^2$ quadrats per replicate and hand-clipped to ground level. Morphology, tiller density, and tiller demographics were determined for each population. Tillers were morphologically classified to quantify MSC and tiller density for each population [11]. Aboveground biomass was determined the week following tiller sampling by cutting and weighing a 0.91-m-wide swath the length of each plot using a flail-type plot harvester (Carter Manufacturing, Brookston, IN) at a 10-cm cutting height. Harvested material was weighed fresh in the field. Subsamples were hand-collected before harvest from each plot, weighed, dried at 50˚C to a constant weight in a forced-air oven, and reweighed to determine dry matter (DM) content. All biomass is reported on a DM basis. Dried samples were ground to pass a 2-mm screen in a Wiley mill and a 1-mm screen in a cyclone mill (Thomas-Wiley Mill Co., Philadelphia, PA).

## 2.2. Switchgrass

Switchgrass populations were planted at the University of Nebraska ENREEC near Mead, NE (41˚09'16"N; 96˚25'30"W) on a Sharpsburg silty clay loam soil. Plots (1.5 × 3.1 m) were seeded into a clean seedbed on 7 June 2002 at 430 pure live seed $m^{-2}$ in rows spaced 15 cm apart with a no-till plot drill as a randomized complete block with six replicates per population. Plots were separated by a 1.5 m wide alley seeded to switchgrass. The seeded plots were sprayed with a tank mixture of quinclorac (Paramount®; 3,7-Dichloro-8-quinolinecarboxylic acid) at 0.56 $kg \cdot ha^{-1}$ and atrazine at 1.1 kg a.i. $ha^{-1}$ pre-emergence after planting for establishment year weed control. No data was collected in the establishment year. The plots were burned on 11 April 2003 and fertilized in late April with 112 kg N $ha^{-1}$ as ammonium nitrate. Herbicides for post-establishment year weed control included a single May application of a tank mixture of metolachlor (Dual II®; 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide at 1.4 kg a.i. $ha^{-1}$, atrazine at 1.1 kg a.i. $ha^{-1}$, and 2,4-D [(2,4-dichlorophenoxy)acetic acid] at 1.1 kg a.i. $ha^{-1}$. In 2004 and 2005, the plots were mowed in early spring to remove re-growth from the previous year. The same post-establishment herbicide treatment was used in 2004. No herbicides were applied in 2005. Stand frequencies were determined in 2002, 2003, and 2006 using a frequency grid [10] at spring green-up. Plots were harvested on 21 August, 9 August, and 8 August in 2003, 2004, and 2005, respectively. Switchgrass plots were harvested, sampled, and samples processed using the same procedures as for big bluestem.

## 2.3. Forage Quality

Big bluestem samples were analyzed in triplicate for *in vitro* dry matter digestibility (IVDMD) with the ANKOM Rumen Fermenter (ANKOM Technology Corp., Fairport, NY) and in duplicate for neutral detergent fiber (NDF) and acid detergent fiber (ADF) with the ANKOM Fiber Analyzer (ANKOM Technology Corp., Fairport, NY) using the procedures described by Vogel *et al.* [12]. Crude protein concentration (CP: %N × 6.25) was determined by the LECO combustion method (Model FP 428 and FP 2000, LECO Corp., St. Joseph, MI) [13] [14].

Switchgrass samples were scanned on a near-infrared reflectance spectrophotometer (NIRS; Model 6500, Silver Spring, MD) and a switchgrass NIRS calibration set was used to predict forage quality parameters. The scans fit within the range of this global calibration set. The calibration samples for the global calibration were chosen by cluster analysis of the reflectance data [15]. Calibration samples were analyzed in triplicate for IVDMD with the ANKOM Rumen Fermenter and in duplicate for NDF and ADF with the ANKOM Fiber Analyzer (ANKOM Technology Corp., Fairport, NY) using the procedures described by Vogel *et al.* [12]. The CP concentration was determined by the same LECO combustion method. Laboratory means were used to develop NIRS calibration equations by partial least squares [15].

Data from big bluestem and switchgrass studies were analyzed using PROC GLM [16]. Strains were considered fixed effects while locations and years were considered random effects. Fisher's protected least significant difference (LSD) was used to separate means of significant effects at $\alpha = 0.05$.

## 3. Results and Discussion

Long-term average annual precipitation is 705 mm at Clay Center, 692 mm at Concord, and 707 mm at Mead, with about 75% occurring during the growing season. Annual precipitation in 2001 was 792 mm at Clay Center, 902 mm at Concord, and 695 mm at Mead. In 2002, the study sites deviated below the long-term average precipitation by 109, 45, and 116 mm for the period from 1 January to 1 July at Clay Center, Concord, and Mead, respectively, with Mead receiving 229 mm by July 1 (**Figure 1**). Big bluestem and switchgrass stand establishment was

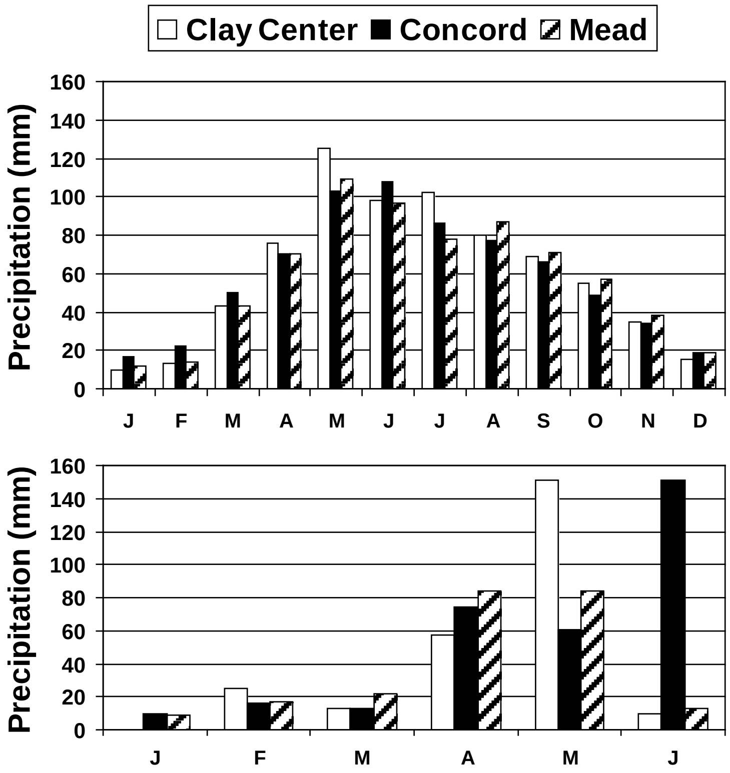

**Figure 1.** Long-term (30-year) average precipitation (top) and monthly precipitation from January through June 2002 (bottom) at Mead, Clay Center, and Concord, NE. January through June precipitation in 2002 was 256, 325, and 229 mm for Mead, Clay Center, and Concord, respectively.

excellent in all environments, with frequency of occurrence of 90% for big bluestem and 84% for switchgrass which fully met the successful stand criteria for these grasses as measured with a frequency grid [10] [17].

### 3.1. Big Bluestem

The biomass yield of all the Pawnee-derived populations selected for seedling traits had significantly lower biomass yields than Pawnee in both the July and August harvests in both sets of experiments averaged over the three locations (**Table 1**). The Pawnee C3SW and Pawnee ST populations had greater regrowth yields than the parent Pawnee. The Pawnee ST and MT strains did not differ for biomass yield in the two-harvest set of trials for the July harvest or the regrowth harvest. The Pawnee MT strain did have 0.5 Mg ha$^{-1}$ greater biomass yields than the Pawnee ST population in the single August harvest set of trials averaged over the three locations (**Table 1**). The base population for the tiller number breeding work, Pawnee C3SW, was intermediate to the MT and ST strains for biomass yield. There were no differences among the strains in the single harvest set of trials harvested in August for forage quality traits (**Table 1**). In the two-harvest set of trials, the Pawnee ST strain had significantly greater IVDMD than the Pawnee MT strain for both the July and regrowth harvest after a killing frost. In this set of trials, the Pawnee ST strain tended to have lower NDF, ADF, and ADL than the Pawnee MT strain but the differences were not significant. Stand frequency

**Table 1.** Biomass yield and quality of Pawnee big bluestem and experimental strains bred for differences in seedling tiller number evaluated in swards in two sets of small plot trials at three locations in eastern Nebraska in 2001 and 2002. One set of trials had a July harvest before heading followed by a late autumn regrowth harvest after frost while the other set of trials had a single harvest in August after heading.

| Strain | Yield | IVDMD | NDF | ADF | ADL | N | Yield | IVDMD | NDF | ADF | ADL | N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mg ha$^{-1}$ | g·kg$^{-1}$ | g·kg$^{-1}$ | g·kg$^{-1}$ | g·kg$^{-1}$ | g·kg$^{-1}$ | Mg ha$^{-1}$ | g·kg$^{-1}$ | g·kg$^{-1}$ | g·kg$^{-1}$ | g·kg$^{-1}$ | g·kg$^{-1}$ |
| | July harvest | | | | | | Regrowth harvest (2002 only) | | | | | |
| Pawnee | 9.2 | 632 | 697 | 378 | 47 | 14 | 1.9 | 504 | 725 | 403 | 54 | 9 |
| Pawnee C3SW | 8.7 | 640 | 688 | 372 | 46 | 14 | 2.4 | 499 | 726 | 408 | 56 | 9 |
| Pawnee ST | 8.6 | 642 | 689 | 372 | 46 | 13 | 2.3 | 510 | 722 | 400 | 54 | 9 |
| Pawnee MT | 8.4 | 628 | 691 | 375 | 47 | 13 | 2.1 | 497 | 729 | 406 | 54 | 9 |
| LSD (0.05)† | 0.4 | 6 | 4 | 4 | 1 | 0.4 | 0.4 | 17 | 8 | 9 | 3 | 1 |
| Single harvest in August | | | | | | | | | | | | |
| Pawnee | 7.4 | 508 | 721 | 397 | 55 | 11 | | | | | | |
| Pawnee C3SW | 6.6 | 506 | 725 | 401 | 56 | 11 | | | | | | |
| Pawnee ST | 6.3 | 508 | 724 | 402 | 57 | 11 | | | | | | |
| Pawnee MT | 6.8 | 500 | 725 | 401 | 56 | 11 | | | | | | |
| LSD (0.05)† | 0.4 | 8 | 4 | 4 | 1 | NS | | | | | | |

†Fisher's protected least significant difference (LSD). The F test for strains in the three-location analysis was significant at $P < 0.05$ for all evaluated yield and quality variables except where NS (not significant) is listed for the LSD.

data was not collected on these trials because excellent stands were obtained on all plots and the stand frequency was greater than the 50% threshold levels above which yield responses to increases in stand density do not occur [18] [19]. Tiller demographic analysis at the July harvest indicated that when evaluated in swards, the Pawnee ST and MT strains did not differ for maturity as measured by MSC, in total tiller density, or in vegetative tiller density (**Table 2**). Although the Pawnee MT population had numerically more of both classes of tillers than the Pawnee ST population, the differences were not significant (**Table 2**). Both populations bred for differences in seedling tiller number had significantly lower total tiller density and vegetative tiller density than Pawnee from which they were derived averaged over the three locations in 2002 (**Table 2**).

Breeding for seedling tiller number in the Pawnee C3SW population which changed seedling tiller numbers in the greenhouse and mature plant differences in tiller number in space-transplanted nurseries did not result in consistent differences in biomass yield or quality when evaluated in multi-location sward trials with two different harvest treatments. Combined with previous breeding work for seedling weight in the development of the Pawnee C3SW population, the combined breeding work for greater seedling weight and tiller traits resulted in a significant reduction in biomass yield when evaluated in swards. This demonstrates the importance of sward evaluation to determine the effectiveness of breeding for specific traits in big bluestem and their effect on other traits.

### 3.2. Switchgrass

Biomass yields were not different among the three Pathfinder strains developed by breeding for seedling traits for the three years of the study (**Table 3**). Averaged over the three harvest years, the strains selected for seedling traits had lower biomass yield than Trailblazer but the differences were not consistent or significant. Trailblazer replaced Pathfinder as the recommended upland cultivar for use in pastures in the region at the time this trial was conducted. The Pathfinder ST and MT strains were lower in IVDMD than the Pathfinder C3SW base population from which they were derived (**Table 3**). The Pathfinder strains selected for seedling tiller number had greater ADF and ADL values than the Pathfinder

**Table 2.** Mean stage count (MSC), total tiller density, and vegetative tiller density for Pawnee, Pawnee single tiller (PST), and Pawnee multiple tiller (PMT) big bluestem grown in established swards at three Nebraska locations in 2002.

| | MSC | Total tillers | Vegetative tillers |
|---|---|---|---|
| | | no. $m^{-2}$ | no. $m^{-2}$ |
| Pawnee | 1.43 | 1200 | 1088 |
| Pawnee ST | 1.54 | 963 | 801 |
| Pawnee MT | 1.47 | 985 | 877 |
| Prob. > F | 0.09 | 0.04 | 0.02 |
| LSD (0.05) | 0.10 | 200 | 197 |

**Table 3.** Mean biomass yield, sward stand frequency, and forage quality of upland switchgrass experimental strains selected for single or multiple tillers as seedlings when evaluated in swards. Seedling tiller selected populations were evaluated in a small plot sward trial near Mead, NE. Yield and quality data are reported for 2003, 2004, and 2005.

| Strains | Biomass yield | | | | Sward stand frequency | | | Forage quality (3-year mean) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2003 | 2004 | 2005 | Mean | 2002 | 2003 | 2006 | NDF | ADF | ADL | IVDMD | N |
| | Mg ha$^{-1}$ | | | | % | | | g·kg$^{-1}$ | | | | |
| Trailblazer | 14.4 | 14.5 | 12.9 | 14.0 | 77 | 88 | 91 | 732 | 411 | 55 | 525 | 10 |
| Pathfinder C3SW | 13.0 | 13.1 | 14.1 | 13.4 | 52 | 67 | 84 | 733 | 411 | 53 | 536 | 11 |
| Pathfinder MT | 13.1 | 15.1 | 12.9 | 13.7 | 69 | 84 | 92 | 739 | 422 | 58 | 513 | 10 |
| Pathfinder ST | 13.0 | 14.4 | 13.4 | 13.6 | 55 | 72 | 90 | 735 | 419 | 57 | 521 | 10 |
| LSD (0.05)† | 1.9 | 2.1 | 1.8 | 0.8 | 12 | 13 | 7 | 5 | 6 | 2 | 10 | 1 |

†The LSD values were obtained from the error term for strains from the analysis of variance of the study which was a randomized complete block with six replicates.

C3SW base population which is consistent with their lower IVDMD. In 2002 and 2003, the Pathfinder MT strain had significantly greater stand frequencies than the Pathfinder ST and the base population, Pathfinder C3SW. However, the stand frequency for the strains in both 2002 and 2003 exceeded the threshold stand frequency of 50% above which Schmer *et al.* [17] and Vogel [18] did not find an association between stand frequency and biomass yield in switchgrass. The results from the switchgrass study are consistent with those from the big bluestem trials.

Das *et al.* [20] reported that selecting for multiple tillers would be an effective method to increase biomass yield in both lowland and upland switchgrass. These results contrast with the previously discussed report of Smart *et al.* [8]. Price and Casler [21] reported that in non-competitive space-planted plots, tillering traits in switchgrass can significantly affect biomass yield but that in competitive sward conditions, plant height and leaf area traits are more important determinants of biomass yield. The results of this study support the results of Price and Casler [21].

## 4. Summary

Breeding for seedling tiller mass and number in big bluestem and switchgrass was not an effective method for improving biomass yield or quality when grown in competitive sward conditions. Although the evaluated populations of big bluestem and switchgrass did have differences in tiller number when grown as spaced plants as previously reported, these genetic differences in tillering potential did not result in increased biomass yield in sward conditions. The results indicate that for both big bluestem and switchgrass, once a stand threshold has been achieved in sward conditions, genetic tillering potential has a limited effect on biomass yield, and other traits such as tiller height, mass, or N demand [22] are more important determinants of biomass yield. The results clearly document the importance of sward trials to measure breeding progress in these grasses as well as the effects of specific traits or genes.

## Publication

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

## References

[1] Casler, M.D., Lee, D., Mitchell, R., Moore, K., Adler, P., Sulc, M., Johnson, K., Kallenbach, R., Boe, A., Mathison, R., Cassida, K., Min, D., Zhang, Y., Ong, R. and Sato, T.K. (2022) Biomass Quality Responses to Selection for Increased Biomass Yield in Perennial Energy Grasses. *BioEnergy Research*, **16**, 877-885. https://doi.org/10.1007/s12155-022-10513-2

[2] Mitchell, R.B., Vogel, K.P., Klopfenstein, T.J., Anderson, B.E. and Masters, R.A. (2005) Grazing Evaluation of Big Bluestems Bred for Improved Forage Yield and Digestibility. *Crop Science*, **45**, 2288-2292. https://doi.org/10.2135/cropsci2004.0716

[3] Vogel, K.P., Mitchell, R.B., Klopfenstein, T.J. and Anderson, B.E. (2006) Registration of 'Bonanza' Big Bluestem. *Crop Science*, **46**, 2313-2314. https://doi.org/10.2135/cropsci2006.02.0068

[4] Vogel, K.P., Mitchell, R.B., Klopfenstein, T.J. and Anderson, B.E. (2006) Registration of 'Goldmine' Big Bluestem. *Crop Science*, **46**, 2314-2315. https://doi.org/10.2135/cropsci2006.02.0070

[5] Sebolai, B. (1989) Evaluation of Three Cycles of Stratified Mass Selection for High Seedling Shoot Weight in Three Warm-Season Grasses. Master's Thesis, University of Nebraska-Lincoln, Lincoln.

[6] Smart, A.J., Moser, L.E. and Vogel, K.P. (2003) Establishment and Seedling Growth of Big Bluestem and Switchgrass Populations Divergently Selected for Seedling Tiller Number. *Crop Science*, **43**, 1434-1440. https://doi.org/10.2135/cropsci2003.1434

[7] Smart, A.J., Vogel, K.P., Moser, L.E. and Stroup, W.W. (2003) Divergent Selection for Seedling Tiller Number in Big Bluestem and Switchgrass. *Crop Science*, **43**, 1427-1433. https://doi.org/10.2135/cropsci2003.1427

[8] Smart, A.J., Moser, L.E. and Vogel, K.P. (2004) Morphological Characteristics of Big Bluestem and Switchgrass Plants Divergently Selected for Seedling Tiller Number.

*Crop Science*, **44**, 607-613. https://doi.org/10.2135/cropsci2004.6070

[9] High Plains Regional Climate Center (2003) Automated Weather Data Network. University of Nebraska, Lincoln.

[10] Vogel, K.P. and Masters, R.A. (2001) Frequency Grid—A Simple Tool for Measuring Grassland Establishment. *Journal of Range Management*, **54**, 653-655. https://doi.org/10.2307/4003666

[11] Moore, K.J., Moser, L.E., Vogel, K.P., Waller, S.S., Johnson, B.E. and Pedersen, J.F. (1991) Describing and Quantifying Growth Stages of Perennial Forage Grasses. *Agronomy Journal*, **83**, 1073-1077. https://doi.org/10.2134/agronj1991.00021962008300060027x

[12] Vogel, K.P., Pedersen, J.F., Masterson, S.D. and Toy, J.J. (1999) Evaluation of a Filter Bag System for NDF, ADF, and IVDMD Forage Analysis. *Crop Science*, **39**, 276-279. https://doi.org/10.2135/cropsci1999.0011183X003900010042x

[13] Watson, M.E. and Isaac, R.A. (1990) Analytical Instruments for Soil and Plant Analysis. In: Westerman, R.L., Ed., *Soil Testing and Plant Analysis*, 3rd Edition, Soil Science Society of America, Madison, 691-704. https://doi.org/10.2136/sssabookser3.3ed.c26

[14] Bremner, J.M. (1996) Nitrogen-Total. In: Sparks, D.L., *et al.*, Eds., *Methods of Soil Analysis*: *Part* 3 *Chemical Methods*, SSSA, Madison, 1085-1121. https://doi.org/10.2136/sssabookser5.3.c37

[15] Shenk, J.S. and Westerhaus, M.O. (1991) Population Definition, Sample Selection, and Calibration Procedures for Near Infrared Reflectance Spectroscopy. *Crop Science*, **31**, 469-474. https://doi.org/10.2135/cropsci1991.0011183X003100020049x

[16] SAS Institute, Inc. (1999) SAS/STAT User's Guide. SAS Institute, Inc., Version 8, Cary, NC.

[17] Schmer, M.R., Vogel, K.P., Mitchell, R.B., Moser, L.E., Eskridge, K.M. and Perrin, R.K. (2006) Establishment Stand Thresholds for Switchgrass Grown as a Bioenergy Crop. *Crop Science*, **46**, 157-161. https://doi.org/10.2135/cropsci2005.0264

[18] Vogel, K.P. (1987) Seeding Rates for Establishing Big Bluestem and Indiangrass with Preemergence Atrazine Applications. *Agronomy Journal*, **79**, 509-512. https://doi.org/10.2134/agronj1987.00021962007900030021x

[19] Masters, R.A. (1997) Influence of Seeding Rate on Big Bluestem Establishment with Herbicides. *Agronomy Journal*, **89**, 947-951. https://doi.org/10.2134/agronj1997.00021962008900060016x

[20] Das, M.K., Fuentes, R.G. and Taliaferro, C.M. (2004) Genetic Variability and Trait Relationship in Switchgrass. *Crop Science*, **44**, 443-448. https://doi.org/10.2135/cropsci2004.4430

[21] Price, D.L. and Casler, M.D. (2014) Predictive Relationships between Plant Morphological Traits and Biomass Yield in Switchgrass. *Crop Science*, **54**, 637-645. https://doi.org/10.2135/cropsci2013.04.0272

[22] Casler, M.D., Lee, D.K., Mitchell, R.B., Adler, P.R., Sulc, R.M., Johnson, K.D., Kallenbach, R.L., Boe, A.R., Mathison, R.D., Cassida, K.A., Min, D.H. and Moore, K.J. (2019) Nitrogen Demand Associated with Increased Biomass Yield of Switchgrass and Big Bluestem: Implications for Future Breeding Strategies. *BioEnergy Research*, **13**, 120-131. https://doi.org/10.1007/s12155-019-10081-y

## Abbreviations

ADF: acid detergent fiber;
IVDMD: *in vitro* dry matter digestibility;
MSC: mean stage count;
MT: multiple tillers;
NDF: neutral detergent fiber;
ST: single tiller.