Characterization of the Leaf Anatomy of Panicum Virgatum Germplasm

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CHARACTERIZATION OF THE LEAF ANATOMY OF PANICUM VIRGATUM GERMPLASM

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ABSTRACT

Both the yield and digestibility of C_4 grasses have been related to anatomical characteristics. The objectives of the current work were to test switchgrass with varied yield and digestibility for variation in anatomical measurements and to develop a method of graphically representing the anatomy. Six accessions were established in replicated field plots and the lamina were sampled, dehydrated and embedded for anatomical study. Midrib dimensions were excluded from study but perimeters of primary and secondary vascular bundles in the lamina were measured. Lamina thickness was determined at the primary bundle, secondary bundle and at the minimum between the bundles. The distance between the primary and secondary bundles was also measured. The accessions differed in perimeter of primary vascular bundles, lamina thickness at the primary bundle and the distance from primary to secondary vascular bundle ($P<0.05$). Variation in lamina thickness at the secondary vascular bundles approached significance ($P<0.10$).

KEYWORDS

Switchgrass, vascular bundles, warm-season, C_4

INTRODUCTION

Switchgrass is a perennial grass with C_4 physiology and anatomy. Switchgrass is native to most parts of North America. Although switchgrass is native to the southeastern United States, relatively little effort has been made to breed improved cultivars for this region (Talbert *et al*., 1983). The high yield potential and lower digestibility of C_4 grasses have both been related to anatomical characteristics such as the cross sectional area of bundle sheath (Wilson, 1993). Yield and digestibility were not significantly correlated in a population of switchgrass and provided plant material for the current study (Talbert *et al*., 1983). The objective of the current work was to determine if switchgrass selected for its variation in yield and digestibility would also exhibit significant variation in anatomy. A secondary objective was to develop a method of describing and graphically representing the differences among the selected plant accessions that facilitated comparisons.

MATERIALS AND METHODS

Six accessions representing a wide range in both digestibility and yield were selected from a previous study (Talbert *et al*., 1983) and established using vegetative material in field plots approximately 1.54-m wide and 6.15-m long with plants on 0.31-m centers. From each plot in four field replications of each accession, two fully expanded lamina were removed and approximately 0.01-m samples were collected from the center of the lamina, fixed in a formalin solution, dehydrated using standard solutions of ethanol and butanol and then vacuum embedded in paraffin. Two slides were made with 5 to 10 microtomed sections (16 m) per slide from each of the 2 samples per plot.

From each slide of the lamina sections, ten images were selected at random (with damaged sections eliminated) for data collection. Since more than 75 % of the lamina dry matter is represented by the lamina excluding the midrib, the midrib was not characterized in this study (Wilson, 1993). Large (primary) and small (secondary) vascular bundles regularly were found arrayed in the lamina in the ratio of 3 or 4 secondary to each primary bundle for all 6 accessions. Therefore, each of the 10 lamina images consisted of pairs of primary and secondary vascular bundles. Since no significant differences in the primary to secondary vascular bundle ratio were detected, this approach was assumed to be representative of the lamina. A number of measurements were made (on the digitized image) using a commercially available image analysis system (Olympus, Lake Success, NY 11042-1179) to characterize certain aspects of the anatomy (Fig. 1). The lamina thickness was measured at 3 positions; first, at the imaged primary bundle, secondly, at the smallest point between the imaged primary and secondary bundles, and thirdly, at the imaged secondary bundle. The perimeter of each bundle and the distance between the bundles was also measured.

Data were analyzed using analysis of variance and means tested with the Waller-Duncan k-ratio t-test with a k-ratio of 100. The experimental units were the 24 field plots comprised of the 6 treatments and 4 replicates and, therefore, the statistical model contained terms for replicates and treatments. Multiple samples (leaves, sections, and images) were averaged prior to analysis of variance.

RESULTS AND DISCUSSION

Switchgrass accessions differed significantly in the lamina thickness at the primary vascular bundles, the perimeter of the primary vascular bundles, and the distance between the primary and secondary vascular bundles (Table). The perimeter of the primary bundles was the most consistent character (CV of 5%) distinguishing the accessions. The perimeter of the primary vascular bundles of 201-4 were 23% greater than those of 131-4. These two accessions also differed in the thickness of the lamina measured at the primary vascular bundles. However, accessions 120-4 and 129-1 had similar perimeters at the primary vascular bundle but the lamina thickness of 120-4 was greater than 129-1. The interbundle distance of 155 m for 201-4 was 28% larger than for 131-4. The interbundle distances of 201-4 and 120-4 were similar even though the perimeters of their primary vascular bundles differed. No significant differences were detected in interbundle lamina thickness, either lamina thickness or perimeter at the secondary vascular bundles. Because of the inherent variability and sampling problems with anatomical measurements, additional sampling may be required to improve statistical power. Logistical constraints may prevent sampling schemes that would detect differences in secondary bundle characteristics.

Proportional diagrammatic representations of the anatomy of the leaf away from the midrib provide a visual basis for comparison of accessions (Fig. 1). The differences and similarities discussed above are illustrated by the diagram. For example, accessions 201-4, 120-4, and 235-2 have similar lamina thickness at the primary vascular bundle but 201-4 has a greater primary vascular bundle perimeter than either accession 120-4 or 235-2. The overall size difference between 201-4 and 131-4 is apparent.

Additional work is planned that will expand the anatomical measurements to include other accessions and attempts will be made to correlate anatomical measurements with yield and digestibility.

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Table 1

Anatomical measurements of the proportions of both primary and secondary vascular bundles (VB), lamina thickness (LT), and VB spacing of switchgrass.

Accession	Primary VB Perimeter	Primary VB LT	Interbundle Distance	Interbundle LT	Secondary VB Perimeter	Secondary VB LT
	μ					
201-4	370	201	155	122	160	171
131-4	301	173	126	97	126	146
120-4	329	192	153	118	160	166
235-2	317	190	148	118	173	166
103-3	319	180	143	115	147	154
129-1	322	171	121	96	106	145
P>F	0.001**	0.020*	0.026*	0.210	0.169	0.099
MSD[a]	25	20	26	ns	ns	ns
CV	5	7	11	16	25	10

[a] MSD = minimum significant difference according to the Waller-Duncan k-ratio t-test with k-ratio = 100.

Figure 1

Diagramatic representation of the variation in anatomy among Panicum vigatum accessions based on threee measurements of lamina thickness, primary and secondary vascular bundle perimeter, and distance from primary to secondary vascular bundles.