Identifying Forage Quality Eastern Gamagrass [*Tripsacum dactyloides* (L.) L.] Genotypes from a Wild Regional Collection

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Abstract
Eastern gamagrass is a perennial warm-season grass native to North America and endemic to the eastern United States. The species is highly valuable as both a forage and hay crop. In 2012, 171 wild-type eastern gamagrass accessions were collected from the southeast, mid-Atlantic and Atlantic coast regions. Each accession was relocated to Starkville, MS (33.423585, -88.792394) and established in a long-term nursery. Accessions were analyzed for ploidy level and during 2013-2014 were further evaluated for desirable forage characteristics including: cold tolerance, delayed maturity, rust resistance, and digestibility. Fourteen elite individuals were identified from the original collection and were propagated for further research. Elite genotypes were divided into individual proaxes and transplanted into a RCB design with three replications. Plots measured 3.04 m x 1.21 m with five replicate plants evenly spaced within the plot. Following a one-year establishment period, whole plots were harvested on a 28-day cycle from May to October. Plots were harvested to a 15 cm stubble height with a Wintersteiger Cibus S harvester. Following each harvest, nitrogen fertilizer was applied to all plots at 56 kg N ha^{-1} using urea ammonium sulfate (32-0-0-12S). Homogenized subsamples were taken to determine percent dry matter, neutral detergent fiber (NDF), acid detergent fiber (ADF), and in-vitro dry matter digestibility (IVDMD). The commercial cultivar 'Highlander', was included in the study as a check. Seasonal yields ranged from 1.19 - 2.73 Mg ha^{-1}. Three accessions – originally collected in Alabama and North Carolina – produced significantly greater forage yield than the check ($P > 0.0001$). Digestibility of the commercial check as well as one accession – collected in Tennessee – were significantly greater than all other accessions ($P > 0.0001$).

Introduction
Eastern gamagrass is primarily used as a forage to be grazed but is also useful as hay or silage (Salon and Cherney 1999). Proper management is vital to successfully maintaining gamagrass, as livestock will often overgraze due to its high palatability. Overgrazing by livestock is believed to be the initial cause of declining natural stands of eastern gamagrass (Rechenthin 1951). Many current commercially available cultivars were originally collected from wild stands in the Midwest region of the United States. At the initiation of this research, no extensive collections of gamagrass germplasm had been conducted throughout the southeast. The original collection described herein was conducted in order to assemble a diverse collection of wild-type germplasm adapted to the climate of the southeast United States. Gamagrass can be either diploid or tetraploid, with diploid being highly desirable for breeding due to the possibility of sexual recombination. This is not possible in tetraploid genotypes as they are predominantly apomictic. It is hypothesized that most native diploid stands of gamagrass are found in the Great Plains region of the United States while most stands found in the southern U.S. are believed to be tetraploid. Diploid populations have been confirmed in Texas and Florida (Newell and de Wet, 1974). This study was designed to identify any potential accessions that possess the necessary characteristics to be a high forage quality cultivar including; cold tolerance, digestibility, rust tolerance and delayed maturity.

Methods and Study Site
For this experiment, from April 2012 to August 2012, 171 accessions of eastern gamagrass were collected from wild native stands across eight states in the southeast, mid-Atlantic, and Atlantic coast regions. Proaxes were harvested from the native stands and transported to Mississippi State University (Starkville, Mississippi). The samples were then transplanted into a nursery block with a 1m x 1m grid arrangement at the Henry H. Leveck Animal Research (South) Farm (33.423756, -88.791594). During 2013 and 2014, evaluations were conducted via visual ratings on all individuals in nursery for desirable forage characteristics such as disease resistance, cold hardiness, and onset of reproduction. Seven individuals failed to survive the winter following

the 2014 growing season. From 28 October to 1 November 2014, ratings for foliar disease susceptibility were determined visually, the main pathogen of interest being southern corn rust (*Puccinia polysora*). Plants were assigned a rating of 1 to 5: 1) No disease present 2) Very few, faint lesions totalling less than 25% coverage of plant 3) Moderate rust infestation, total coverage not exceeding 50% of plant material 4) Abundant rust and fungal lesions, covering more than 50% of leaf tissue 5) Complete infestation, entire plant displaying rust spores or fungal lesions.

The next screening conducted was for cold hardiness. On two separate dates, 20 November and 5 December, individuals were evaluated 5 days following overnight ambient air temperatures of -5°C. Plants were assigned a rating of 1 to 5: 1) Severe leaf tissue damage, complete loss of tissue structure 2) Widespread leaf tissue damage and loss of color to greater than 50% of total leaf area 3) Moderate tissue damage and loss of color on up to 50% of total leaf area 4) Minimal leaf tip damage and some loss of color to less than 25% of total leaf area 5) No presence of damage to leaves.

The final visual assessment was onset of reproduction, which was defined as the first visible reproductive tiller with an emerged inflorescence. These ratings were taken across May and June and broken down on a weekly basis (Week 1= 15 – 22 May, Week 2= 22 – 29 May, Week 3 = 29-May – 5-June, Week 4 = 5-June – 12-June, Week 5= 12-June – 19-June, Week 6 = 19-June – 26-June).

Forage quality was assessed in separate harvest events in 2014 and 2015. Entire plants were collected using a handheld electric sickle bar type harvester leaving behind a stubble height of 30 cm. Whole plants were homogenized, divided into subsamples and analyzed. Subsamples were dried in a forced air oven at 60°C for 96 hours, then ground to pass a 2 mm mesh in a Thomas® model 4 Wiley® mill (Thomas Scientific, Swedesboro, NJ). Samples were processed for IVTDMD according to ANKOM Technology Dietary Fiber Analysis Method 3 using the DAISY^{II} Incubator (ANKOM Technology, Macedon, NY). All samples were analyzed in duplicate at the Mississippi State University H.W. Essig Nutrition Lab in the James W. Scales Building (Mississippi State, MS).

Results

Rust Resistance

Visual ratings for rust resistance were significantly affected ($P< 0.0001$) by individuals in the population. Mean raw data values ranged from 5.0 – 1.0 (LSD = 0.9278), with an overall raw mean of 2.9 and a median of 3. Mississippi accessions were among the most severely infested individuals in the entire collection. Of the lowest ranked (highest rust infestation) mean separation, Mississippi accessions comprised 71% (10 of 14) of that group, while the highest ranked (lowest rust infestation) mean separation group is 35% (7 of 20) Mississippi accessions.

Cold Tolerance

Evaluation of cold tolerance ratings showed that genotype had no significant effect ($P=0.7264$) on cold tolerance rating (LSD=2.168). Mean cold tolerance ratings ranged from 1.0 to 4.0, with a mean rating of 2.5 and 1.3 in November and December, respectively. Median rating for November was a 3, while December was 1.

Onset of Reproduction

Onset of reproduction began in May and continued through June. By the 5th week of June, all entries had initiated reproduction. Overall mean date of initiation of reproduction was week 3.07. Results were divided by state of origin and week of maturity initiation. A total of 21 entries were collected from the state of Alabama. Of those individuals, mean flowering date occurred later (0.93 weeks) than the overall population mean date of initiation. Accessions from Arkansas (5) had a mean date of initiation of reproduction of 1.8 weeks, 1.27 weeks earlier than the overall population mean. Georgia accessions (16) had a mean date of initiation of reproduction of 3.125 weeks, nearly identical with the overall population mean. The single Kentucky individual began reproduction in week two (22-29 May). Individuals collected from Mississippi (96) had a mean initiation of reproduction of 2.85 weeks, with over 50% of those individuals becoming reproductive within the first two weeks of observation. North Carolina (5) and South Carolina (10) accessions were later maturing than most other collections, averaging 3.8 and 4.1 weeks to maturity, respectively. Lastly, Tennessee accessions (9) had a mean onset of maturation date of 2.1 weeks, much earlier than the overall population mean.

Forage Digestibility

Individual plants in nursery showed significant differences ($P < 0.0001$) in forage digestibility, shown as IVTDMD (LSD = 2.67). IVTDMD of forage samples ranged from 50.2% - 76.3% with an overall mean of 64.5 and a median of 65.2. Of the 14 elite genotypes, the accession from Tennessee showed significantly higher IVDMD compared to the others and equal to the commercial check.

When looking at yield for the elite 14, all accessions had comparable yields to the commercial check, with three accessions, two from Alabama and one from North Carolina showing yields significantly greater than 'Highlander'.

Discussion [Conclusions/Implications]

From these, 14 individuals were determined to be elite and were propagated for further research, along with a check of the commercial variety Highlander.

Despite notable differences in phenotypic characteristics, all collected accessions were determined to be tetraploid. Morphological characteristics such as leaf width, leaf color and earliness of spring growth that have been used previously to differentiate diploid individuals from tetraploid individuals (Dunfield 1986) could not be used for stands located in the southern and Atlantic regions.

From the 14 selected elite genotypes, five were originally from Mississippi, three from Alabama, two from South Carolina, one from each of Georgia, North Carolina, Arkansas and Tennessee. Of the 14 elite genotypes, the accession from Tennessee showed significantly higher IVDMD compared to the others and equal to the commercial check. When looking at yield for the elite 14, all accessions had comparable yields to the commercial check, with three accessions, two from Alabama and one from North Carolina showing yields significantly greater than 'Highlander'.

The complete lack of any diploid germplasm in this collection supports the long-held hypothesis that naturally occurring wild-type stands throughout the southeast are predominantly tetraploid. While the absence of diploid germplasm from this collection also makes selection breeding difficult, some sexual recombination is achieved in tetraploid gamagrass, preserving the possibility of genetic improvement (Dewald and Kindiger 1998).

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