# Perennial Grass Breeding Program for Forage and Bio-fuels – Tifton, GA

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The perennial grass breeding program at Tifton, Georgia has a long history. Drs. Glenn Burton and Wayne Hanna, with collaborators, developed a significant number of forage and turf cultivars over the past 60-plus years. Work is continuing in areas of forage and turf improvements for the South. Forage improvement of bermudagrass and bahiagrass continues within the Crop Genetics and Breeding Research Unit of USDA/ARS. Recently, a new effort has begun within the unit toward developing perennial grass crops as feedstocks for bio-energy in the Southeast.

An emphasis beginning three years ago in the forage breeding program was to evaluate and reestablish the 600 plus forage bermudagrass (*Cynodon* spp.) plant introduction nursery. Accessions in this nursery have been accumulated from Africa, Asia, Europe, and the United States and from previous breeding efforts over the past 60 years. It is from this material that Dr. Burton screened and crossed material with high digestibility, fall armyworm tolerance and high yields to produce hybrids such as Coastal, Tifton 44 and Tifton 85. A reevaluation of the material was begun by assessing phenotypic traits such as plant height, leaf width and length, coarseness of stems as well as ploidy levels by use of a flow cytometer. The entire nursery was re-established first in pots in the greenhouse then by replanting at a new location in the spring of 2004. These plots underwent further evaluation for traits such establishment rate, stolon number and length (Table 1). From the entire collection a core collection of 170 genotypes was developed using clustering analysis of fourteen phenotypic traits and ploidy levels (Anderson, 2005). The core collection is currently being evaluated for *in vitro* digestibility, fall armyworm resistance, shade tolerance and chemical attributes. Amplified fragment length polymorphisms (AFLP) have indicated great amounts of genetic variability that may be used for development of molecular markers to assist breeding for important traits.

Synthetic seeded breeding lines and a vegetatively propagated hybrid bermudagrass are being evaluated for release along with a fast germinating bahiagrass. Seeded forage bermudagrass with cold tolerance continue to be goals for forage improvement within the breeding program. Agronomic studies that involve fertilization rates and sod based rotations are also underway.

A greater emphasis has been placed on evaluating perennial grasses as feedstocks for conversion to bio-energy or bio-fuels. The sugar-based fermentation conversion of ligno-cellulosic plant material to ethanol has been researched extensively over the past few decades. The premise is to convert as much of the dry matter cellulose and hemi-cellulose as possible to hexoses and pentoses. Highly efficient cellulases and hemi-cellulases are being commercially developed, however, complex cell-wall structures that have cellulose and hemi-cellulose bound to lignin restrict access of these enzymes. For that reason, it is necessary to evaluate germplasm for traits that make conversion more efficient, then breed or engineer plants with lower lignin or with cell-wall structures more amenable to decomposition. Digestion by rumen microbes correlates very well with desirable traits for conversion to ethanol (Table 2) (Anderson et. al., 2005).

Thermo-chemical processes such as pyrolysis and gasification are also methods of converting ligno-cellulosic plant material to useable energy. In this case high biomass yields combined with lower ash and silicon content are desireable. A multi-species test has been established to determine comparative biomass yields, nutrient cycling, and carbon sequestration. Napiergrass (*Pennisetum purpurem*), giant reed (*Arundo donax*), and bermudagrass are among the species under evaluation as potential dedicated crops for bio-energy in the Southeast.

References:

Anderson, W. F. 2005. Development of a forage bermudagrass (*Cynodon* sp.) core collection. Grassland Sci. 51:305-308.

Anderson, W.F., J. Peterson, D.E. Akin, and W.H. Morrison, III. 2005. Enzyme pretreatment of grass lignocellulose for potential high-value co-products and an improved fermentable substrate. Appl. Biochem. Biotechnol. 121-124:303-310.

Table 1: Means of 23 phenotypic traits within 11 major clusters from cluster analysis of full bermudagrass germplasm collection at Tifton, GA, taken during the summer of 2003.

| Cluster | Number of entries from nursery | Number of entries in core | Emer-gence rating | Early heading rating | Leaf coarse rating | Plant height (cm) | Head height (cm) | Lodging rating | Plant density rating |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 81 | 23(28%) | **3.33** | 4.62 | **1.89** | **27.5** | 34.6 | 4.14 | 4.43 |
| 2 | 55 | 30(57%) | 2.80 | 4.09 | 4.18 | **64.2** | **66.9** | 2.93 | 4.78 |
| 3 | 76 | 8(9%) | 2.24 | 2.21 | 2.24 | 46.2 | 50.9 | 2.64 | 4.89 |
| 4 | 90 | 26(29%) | **1.75** | 3.20 | 2.36 | 46.6 | 48.8 | 1.89 | 4.90 |
| 5 | 79 | 21(27%) | 2.16 | 3.37 | 2.11 | 27.7 | **34.5** | 4.44 | **3.73** |
| 6 | 61 | 12(20%) | 2.69 | **4.75** | 2.44 | 39.1 | 45.7 | 2.90 | 4.77 |
| 7 | 58 | 14(24%) | 1.84 | 4.34 | 2.36 | 42.1 | 47.4 | 2.81 | 4.81 |
| 8 | 15 | 5(33%) | 2.33 | 3.07 | **4.40** | 54.7 | 60.7 | **4.80** | **3.73** |
| 9 | 31 | 10(32%) | 1.84 | 3.26 | 3.03 | 60.3 | 64.7 | 1.65 | 4.97 |
| 10 | 42 | 15(36%) | 2.38 | 3.85 | 3.76 | 45.8 | 53.1 | 4.36 | 4.02 |
| 11 | 10 | 4(40%) | 2.30 | **0.80** | 2.30 | 52.4 | 0.0 | **1.30** | **5.00** |

| Cluster | Head density rating | Raceme number | Raceme Length (cm) | Leaf length (cm) | Leaf width (mm) | Inter-node length (cm) | Stem thick-ness rating | Fall regrowth rating |
|---|---|---|---|---|---|---|---|---|
| 1 | **4.01** | 4.38 | 4.63 | **6.63** | 2.25 | **1.91** | 1.26 | 3.14 |
| 2 | 2.85 | 5.62 | **7.13** | 15.31 | 4.99 | 4.97 | 2.82 | 3.16 |
| 3 | 2.66 | 4.73 | 5.96 | 8.55 | 2.72 | 3.03 | 1.37 | 2.64 |
| 4 | 2.30 | 4.42 | 4.90 | 8.59 | 2.25 | 3.25 | **1.24** | 3.06 |
| 5 | 3.09 | 4.48 | 4.79 | 7.02 | **2.18** | 2.37 | 1.65 | **2.24** |
| 6 | 3.98 | 4.73 | 5.62 | 8.31 | 2.72 | 3.04 | 1.62 | 3.20 |
| 7 | 3.47 | 4.40 | 5.46 | 8.45 | 2.22 | 3.40 | 1.60 | 2.79 |
| 8 | 2.87 | **7.12** | 6.37 | **17.27** | **6.27** | **6.07** | **4.07** | 3.47 |
| 9 | 3.03 | 4.69 | 6.15 | 9.48 | 2.89 | 4.08 | 1.77 | 2.77 |
| 10 | 3.26 | 5.41 | 6.57 | 12.96 | 4.42 | 4.17 | 2.55 | **3.52** |
| 11 | **0.00** | **0.00** | **0.00** | 9.64 | 2.45 | 3.45 | 1.60 | 3.10 |

| Cluster | Fall plant height | Fall head rating | Regrowth rating 04 | Stolon number 04 | Stolon length 04 | Plant height (cm) 04 | Establishment rating 04 | Head rating 04 |
|---|---|---|---|---|---|---|---|---|
| 1 | 13.5 | 1.79 | 1.83 | 17.8 | 66.4 | 26.9 | 3.80 | **4.10** |
| 2 | **33.5** | **3.20** | **3.89** | 29.9 | 116.9 | **65.6** | **4.87** | 1.24 |
| 3 | 15.1 | 0.29 | 2.64 | 26.2 | 73.0 | 32.0 | 3.99 | 3.07 |
| 4 | 17.9 | 0.37 | 2.72 | 14.9 | 83.8 | 45.3 | 4.09 | 1.52 |
| 5 | **10.7** | 0.84 | 2.13 | 18.1 | **59.1** | **31.4** | 3.82 | 2.51 |
| 6 | 16.0 | 1.37 | 3.02 | **33.3** | 82.4 | 43.6 | 4.56 | 3.16 |
| 7 | 17.5 | 1.09 | **1.75** | **9.7** | 63.0 | 38.7 | **3.03** | 2.98 |
| 8 | 27.3 | 0.20 | 3.60 | 23.2 | **124.0** | 62.0 | 4.73 | **0.33** |
| 9 | 19.0 | 0.74 | 3.23 | 32.9 | 104.7 | 54.1 | 4.71 | 2.87 |
| 10 | 23.7 | 3.17 | 2.55 | 13.5 | 81.3 | 53.3 | 4.05 | 2.67 |
| 11 | 23.3 | **0.00** | 2.50 | 11.5 | 70.1 | 43.2 | 3.60 | 1.00 |

**Table 2**. Percent dry weight (DW) loss, and free sugars released in filtrate after pretreatments with commercial esterase and cellulase for bermudagrass and napiergrass genotypes at 4 and 8 weeks of age compared to *in vitro* dry matter digestibility (IVDMD).

| ***Genotype*** | ***Age*** | ***% DW loss†*** | ***Xylose (mg/g)†*** | ***Glucose (mg/g)†*** | ***IVDMD-leaf‡*** | ***IVDMD -stem‡*** |
|---|---|---|---|---|---|---|
| Coastal (B) | 4 weeks | 39 | 8.8 | 87.1 | 46.01 | 46.18 |
| Coastal (B) | 8 weeks | 41 | 7.5 | 107.4 | 49.75 | 49.25 |
| Tifton 85 (B) | 4 weeks | 50 | 13.7 | 98.2 | 58.53 | 58.74 |
| Tifton 85 (B) | 8 weeks | 42 | 12.4 | 113.1 | 54.84 | 50.17 |
| Tifton 44 (B) | 4 weeks | 38 | 9.8 | 87.8 | 50.43 | 53.61 |
| Tifton 44 (B) | 8 weeks | 34 | 9.5 | 80.3 | 51.62 | 45.21 |
| CC II (B) | 4 weeks | 46 | 14.7 | 111.7 | 58.44 | 53.86 |
| CC II (B) | 8 weeks | 36 | 12.5 | 116.7 | 51.96 | 43.27 |
| Merkeron (N) | 4 weeks | 64 | 19.7 | 125.6 | 68.11 | 64.96 |
| Merkeron (N) | 8 weeks | 46 | 15.9 | 95.1 | 61.55 | 55.01 |

†Values are the sum of subsequent incubations with esterase for 24 h and then cellulase for 72 h.
‡48 hour rumen digestion, 48 pepsin digestion