ORNL/SUB-02-19XSV810C/01

Bioenergy Crop Breeding and Production Research in the Southeast

Final Report for 1996 to 2001

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Environmental Sciences Division

BIOENERGY CROP BREEDING AND PRODUCTION RESEARCH IN THE SOUTHEAST

FINAL REPORT FOR 1996 TO 2001

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Date Published: December 2002

Prepared for
U.S. Department of Energy
Office of Biomass
Budget Activity No. EB 52 03 00 0

Prepared by
OAK RIDGE NATIONAL LABORATORY
Oak Ridge, Tennessee 37831
managed by
UT-BATTELLE, LLC
for the
U.S. DEPARTMENT OF ENERGY
under contract DE-AC05-00OR22725

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FIVE-YEAR PROJECT SUMMARY

Switchgrass (*Panicum virgatum* L.) is a native grass species to much of the United States. It has shown great potential for use in production of fuel ethanol from cellulosic biomass (Lynd et al., 1991). Work in Alabama demonstrated very high dry matter yields can be achieved with switchgrass (Maposse et al. 1995) in the southeastern United States. Therefore, this region is thought to be an excellent choice for development of a switchgrass cropping system where farmers can produce the grass for either biomass or forage. Another report has shown success with selection and breeding to develop high yielding germplasm from adapted cultivars and ecotypes of switchgrass (Moser and Vogel 1995). In the mid 1990s, however, there was little plant breeding effort for switchgrass with a potential for developing a cultivar for the southeast region.

The main goal of the project was to develop adaptive, high-yielding switchgrass cultivars for use in cropping systems for bioenergy production in the southeastern United States. A secondary objective was to assess the potential of alternate herbaceous species such as bermudagrass (*Cynodon dactylon* L.), bahiagrass (*Paspalum notatum* Flugge.), and napiergrass (*Pennisetum purpureum* Schumach.) that may compete with switchgrass for herbaceous bioenergy production in the southeast. During the conduct of the project, another goal of developing molecular markers useful for genetic mapping was added.

Key professional personnel were Joe H. Bouton, Professor, University of Georgia (UGA), Overall Project Principal Investigator; Carl S. Hoveland, Professor, UGA, and Wayne W. Hanna, United States Department of Agriculture, Agricultural Research Service (USDA-ARS), collaborators for field performance studies; and Andrew Paterson, Professor, UGA, collaborator for molecular mapping studies.

The "lowland" cultivars, Alamo and Kanlow, were found to be the highest yielding switchgrass cultivars. Although most summers during the project period were hot and dry, their annual dry matter yield continue to outperform the best "upland" cultivars such as Cave-in-Rock, Shawnee, NE Late, and Trailblazer.

The use of a breeding procedure based on the "honeycomb design" and multi-location progeny testing, coupled with the solid heritability and genetic gain estimates for dry matter yield in lowland type switchgrass germplasm, indicated excellent potential to isolate parental genotypes for producing higher yielding synthetic cultivars. The four experimental synthetics produced thus far, and now in performance tests, could provide this cultivar. Initial performance results of these experimentals have been very promising demonstrating a 30% yield enhancement over Alamo and Kanlow. Future testing, including testing in other states, will be critical before a determination can be made to release one or more of these into the commercial seed trade.

In the genetic mapping project, 42 genotypes of switchgrass were surveyed using restriction fragment length polymorphism (RFLP) probes from different grass species. The different genotypes included 24 from Alamo, 15 from Kanlow, and 3 from "Summer." A majority of the probes (87%) hybridized to the switchgrass DNA and 81% were polymorphic. Most of the polymorphism observed was between the cultivars. A mapping population consisting of 100 progeny from a cross between the most dissimilar Kanlow and Summer genotypes was produced during 2001. The parents and progeny population are now maintained at the University of Georgia and will be used to construct a map based on the polymorphic RFLP probes.

When compared to “Tifton 85” bermudagrass, “Tifton 9” bahiagrass, and “Merkron” napiergrass, Alamo switchgrass was found to show poorer yields than Merkron and Tifton 85, but better yields than Tifton 9 in the coastal plain region. The exceptional performance of Tifton 85 bermudagrass is extremely noteworthy because this hybrid bermudagrass is also a variety of choice for many commercial hay producers in the lower south and would give any producers a very good option to produce either biomass for a biofuels initiative or sell as hay on the open market. Merkron has consistently showed the highest dry matter yields. However, there continues to be some winter damage each year on this species at the Athens location indicating its real potential lies mainly in the Gulf Coast region of the southeastern United States. The excellent characteristic of Tifton 85 and Merkron should therefore be enough to initiate more basic research for their chemical ability as a biofuels crop.

Throughout this project, we continually encountered problems establishing switchgrass directly into non-fumigated soil. Even when plants emerge and establish, their productivity during the initial year was very poor. The reason for this is not readily apparent, but could be partially due to the very hot and dry summer experienced during the project period. Preliminary experiments continue to show a need for using a pre-plant and post-plant herbicide program, especially when using no-till methods, to give more productive stands. Management research to better establish switchgrass will need to continue in the future. Finally, good seedling establishment was also part of the protocol for selecting parents for our experimental cultivars. It is hoped that these new experimentals will have better establishment traits that will be apparent in future trials.

PROJECT PUBLICATIONS

McLaughlin, S., J. Bouton, D. Bransby, B. Conger, W. Occumpaugh, D. Parrish, C. Taliaferro, K. Vogel, and S. Wullschleger. 1997. Evaluating and improving switchgrass as a bioenergy crop. pp. 137–143. In R. P. Overend and E. Chornet (eds.), Biomass in Energy, Environment, Chemicals, Fibers, and Materials. In Proceedings of the Third Biomass Conference of the Americas, Montreal, Quebec, Canada. Aug. 24–29, 1997. Elsevier, N.Y.

McLaughlin, S. B., J. Bouton, D. Bransby, B. Conger, W. Ocumpaugh, D. Parrish, C. Taliaferro, K. Vogel, and S. Wullschleger. 1999. Developing switchgrass as a bioenergy crop. pp. 282–299. In J. Janick (ed.), Perspectives on new crops and new uses. ASHS Press, Alexandria, Va. In Proceedings of the Fourth National Symposium New Crops and New Uses: Biodiversity and Agricultural Sustainability.

Taliaferro, C. M., K. P. Vogel, J. H. Bouton, S. B. McLaughlin, and G. A. Tuskan. 1999. Reproductive characteristics and breeding improvement potential of switchgrass. In Proceedings of the Fourth Biomass Conference of the Americas, Aug. 29–Sept. 2, 1999. Oakland, Calif.

FIVE-YEAR PROJECT REPORT

INTRODUCTION

Switchgrass varieties and ecotypes are classified as either lowland or upland types (Moser and Vogel 1995). Lowland types occur in river bottom areas, are tall and robust, possess a bunch-type growth habit, and are tetraploids (2n=36). Upland types are shorter and finer, possess long rhizomes which allow them to spread more, and are either hexaploids or octoploids (2n=54, 72).

In a six-year study in Alabama, the lowland variety Alamo was found to average 24.5 Mg ha-1 dry matter yield (Maposse et al. 1995). This was 42% more dry matter than another lowland type, Kanlow, but also 135% higher than the average of the six upland varieties in the trial ("Blackwell," "Cave-in-Rock," Kansas Native, "Pathfinder," "Summer," and "Trailblazer"). Although selection and breeding has been successful in developing high yielding germplasm from adapted cultivars and ecotypes of switchgrass (Moser and Vogel 1995), there has been little switchgrass breeding with a potential to improve the crop for the southeast region.

The future for crop improvement for high management systems will surely include using molecular markers and transformation technologies to develop the modern cultivars. Molecular markers from genetic maps are being used in crop improvement programs to increase the efficiency of selection for complex traits. Molecular markers are therefore an important tool for plant breeders during selection.

To-date, molecular studies of switchgrass have been limited to chloroplast DNA variations (Hulquist et al. 1996) and RAPD diversity among different populations (Gunter et al. 1996). We are now participating in developing a linkage map of switchgrass. This low density frame map based initially on RFLPs will be used as starting point to construct a more saturated genetic linkage map which further could permit us to extend comparative genome mapping with other species and genera and the identification of quantitative trait loci (QTLs) for important complex characteristics.

GOALS AND OBJECTIVES

The main goal of the project was to develop adaptive, high-yielding switchgrass cultivars for use in cropping systems for bioenergy production in the southeastern United States. A secondary objective was to assess the potential of alternate herbaceous species that may compete with switchgrass for herbaceous bioenergy production in the southeast. During the conduct of the project, another goal of developing molecular markers and mapping was added. The specific objectives were:

Objective 1. Initiate a breeding program to improve existing switchgrass cultivars for the southeast.

Objective 2. Provide data on yield performance of the best currently available switchgrass cultivars and any new selections on representative soil types of the southeast.

Objective 3. Evaluate the potential of selected alternate herbaceous species that may compete with switchgrass for herbaceous biofuels production in the southeast.

Objective 4. Develop a genetic framework map for switchgrass based on the single dose restriction fragment approach; develop a genetic map that includes RFLP and SSR markers.

RESEARCH RESULTS

Objective 1

The breeding program was initiated to select adaptive, higher yielding cultivars from Alamo and Kanlow. Alamo and/or Kanlow were also used as the best checks to monitor performance of any newly developed experimental cultivar from our program.

Our original breeding effort was initiated by randomly selecting 1000 genotypes from Alamo and Kanlow and space planting these into a honeycomb design (Fasoulas and Fasoula 1995). This design was used because of its ability to remove micro-environmental variation during assessment of individual, un-replicated genotypes. Initial data showed genetic variances for yield were high, especially for Alamo selections, indicating good potential to increase yield within these populations. Based on phenotypic selection using the honeycomb planting design, heritability estimates were found to be adequate to incur good gains from selection and genotypes were then identified from each cultivar which had excellent performance. Cuttings of each of these superior genotypes were clonally multiplied in the greenhouse and used in a polycrossing program to produce half-sib progeny of each and to assess their seed yield. The highest seed yielding genotypes were then progeny tested in sward plots over locations and years and the highest yielding genotypes selected for inclusion into another replicated polycross to generate new experimental synthetic cultivars, GA-991, GA-992, and GA-993. These are therefore based exclusively on parentage from Alamo and/or Kanlow.

Three of the new experimental synthetics produced by this project, GA-991, GA-992, and GA-993, were established in small plot performance trials at the Tifton and Athens testing locations during 2000. First-year data were highly variable due to dry weather and concurrent establishment problems. However, during 2001, full season yields were obtained and showed the high yielding ability and the success of the breeding effort, for these experimentals at both test locations (approximately 30% average yield increase over Alamo and Kanlow).

Entry	Yield (kg ha^{-1})		Average yield (kg ha^{-1})
	Athens	Tifton	
GA-993	12,563	20,791	17,500
GA-991	11,716	21,162	17,384
GA-992	10,540	20,976	16,801
Kanlow	9,755	15,380	13,130
Alamo	6,313	18,088	13,378
L.S.D. ($P < 0.05$)	1,620	3,457	2,067

Another cycle of phenotypic selection using the honeycomb design also allowed new genotypes to be identified Alamo and Kanlow which were also polycrossed, progeny tested over years and locations, and the best ones intermated to form an additional synthetic, GA-001. This experimental was seeded, along with the other 3 experimentals, during 2001 at Tifton and experienced an outbreak of an unknown disease during establishment. This disease greatly damaged the Alamo control and the GA-991 experimental, but GA-992, GA-993, and GA-001 shown high resistance that resulted in good yields for these experimentals by the autumn harvest.

Entry	Autumn harvest (kg ha^{-1})
GA-001	2060
GA-993	1990
GA-992	1415
GA-991	991
Alamo	558
L.S.D. ($P < 0.05$)	904

Objective 2

Concurrent with the breeding program, performance trials were conducted at two distinct locations in Georgia, Tifton and Athens. When summarized across both test locations and over all test years, these trials conclusively demonstrated the superiority of the lowland cultivars, Alamo and Kanlow, over upland cultivars, Cave-in-Rock, Shawnee, NE Late, and Trailblazer, and supported our original decision to base our cultivar improvement program on the lowland germplasm sources.

Variety	Yield (kg ha^{-1})		Average yield (kg ha^{-1})
	Athens	Tifton	
Alamo	16,132	16,324	16,220
Kanlow	15,502	15,948	15,705
Cave-in-Rock	10,519	7,587	9,186
NE Late	10,295	6,120	8,397
Shawnee	9,774	8,067	8,999
Trailblazer	8,790	5,751	7,409
L.S.D. ($P < 0.05$)	1,153	976	1,563

In conjunction with this objective, a trial was sown at Athens to examine different establishment methods (e.g., planting dates, herbicide treatments) under no-tillage conditions for the production of high dry matter yield from the lowland variety Alamo. Experiments were established at two locations (Athens and Tifton) using the following treatments: factorialized combinations of planting dates, early-season (May), mid-season (July), and late season (September), with different herbicide treatments, non-herbicide control, Gramoxone at planting, metsulfuronmethyl (MSMA-Ally) postemergence, and Gramoxone at planting with MSMA-Ally postemergence. These plots were harvested for spring-early summer dry matter yield during the next year with the following results:

Site and treatment	Yield (kg ha^{-1})		
	Early	Mid	Late
Athens:			
Control	2,461	0	0
Gramoxone	8,719	0	0
MSMA-Ally	3,122	0	0
Gramoxone/MSMA-Ally	9,258	0	0
LSD ($p < 0.05$)	1,987	NS	NS
Tifton:			
Control	9	0	0
Gramoxone	1,285	0	0
MSMA-Ally	407	0	0
Gramoxone/MSMA-Ally	1,887	0	0
LSD ($p < 0.05$)	942	NS	NS

These data, therefore, demonstrated that early season planting was critical as no stands were achieved at the mid or late season planting dates at either location. Better stands and yield during the following year were achieved at Athens than Tifton and application of Gramoxone herbicide was critical in achieving productive stands. However, even the best treatments required a year to establish productive stands, and indicate that establishment will continue to be a problem in order for producers to be successful.

Objective 3

In conjunction with Objective 2, the 2001 yield data were collected from the same long term trials described above in Objective 2 and allowed us to compare the performance of "Alamo" switchgrass from that trial with "Tifton 85" bermudagrass, "Tifton 9" bahiagrass, and "Merkron" napiergrass planted adjacent to that trial. The design of this experiment did not allow statistical treatment of the data and only showed overall trends. Napiergrass produced very high dry matter yields compared to the other species.

Species (variety)	Yield (kg ha^{-1})		Average yield (kg ha^{-1})
	Athens	Tifton	
Switchgrass (Alamo)	16,132	16,324	16,220
Napiergrass (Merkron)	27,457	28,070	27,764
Bermudagrass (Tifton 85)	17,623	17,525	17,578
Bahiagrass (Tifton 9)	11,233	10,787	11,030

A separate trial comparing these same varieties at Midville, Georgia, were done in a statistically valid manner. At the Midville testing location, napiergrass continued to be the highest yielding species when averaged for all years in the test. Alamo switchgrass did not perform well in this trial and gave much lower yields than expected based on data from the other test locations.

Species (variety)	Annual yield (kg ha^{-1})
Napiergrass (Merkron)	30,717
Bermudagrass (Tifton 85)	17,560
Bahiagrass (Tifton 9)	11,730
Switchgrass (Alamo)	11,450
L.S.D. ($P < 0.05$)	1,702

However, during 2001, the dry matter yield of napiergrass was greatly reduced compared to the other species, and it actually produced less yield than switchgrass and bermudagrass at all testing locations (see data presented in the 2001 annual report section on page below).

Objective 4

In 1998, a genetic mapping project based on molecular markers switchgrass as added as an additional objective to the project. Since there were no available reports relevant for RFLP analysis in switchgrass, we initially concentrated on finding suitable procedures for nuclear DNA isolation, surveying filters with heterologous grass probes, and developing specific probes for switchgrass. We tested several procedures for DNA isolation on samples of Alamo and Kanlow. The main procedures were based on phenol extraction, cetyl trimethyl ammonium bromide (CTAB), and a mixture of polyvinylpyrrolidon (PVP), sodium dodecyl sulfate (SDS), and LiCl. None of the original methods gave sufficient quantities of DNA needed for RFLP analysis. We finally developed a simple and inexpensive protocol for DNA extraction based on a modified CTAB procedure.

Forty-two genotypes of switchgrass were also surveyed using RFLP probes from different grass species (probes also obtained courtesy of Dr. Andrew Paterson, UGA, Athens, Georgia). The different genotypes included 24 from Alamo, 15 from Kanlow, and 3 from Summer (all Summer and 3 of the Kanlow genotypes obtained courtesy of Dr. Ken Vogel, USDA-ARS, Lincoln, Nebraska). In the course of the survey, 21 genotypes originating from commercial seed (Alamo and Kanlow) were discarded since they kept showing the same banding pattern. Total genomic DNA was extracted from 5 to 10 g of leaves from each genotype using a modified CTAB procedure. Ten ug of DNA were digested with each of four restriction enzymes (EcoRI, EcoRV, HindIII, and XbaI) and fragments separated on 0.8% agarose gels. DNA was then transferred to nylon membranes (Amersham hybond-N+) by capillary blotting in a 0.4 N NaOH buffer. One-hundred twenty-one probes were hybridized to filters containing DNA from the 21 remaining genotypes digested with the four restriction enzymes. These included 22 PCD (bermudagrass), 46 PAP (*Pennisetum*), and 53 RZ (Rice) probes. The filters were then exposed to x-ray films for a period of 1-7 days depending on the signal counts (3000/CPM).

All autogradiographs were scored for the presence or absence of bands. Data from the first 68 probes were analyzed by SYSTAT 10.1 using probes and enzymes as independent variables and genotypes as dependent variables. Hierarchical clustering was used to determine the distance matrix between the different genotypes. Eighty-seven percent of the probes used hybridized to the switchgrass DNA and 81% were polymorphic. Most of the polymorphism observed was between the cultivars. The dissimilarity between Kanlow and Summer ranged from 0.71 to 0.80; between Summer and Alamo 0.64 to 0.85; within Alamo 0.03 to 0.33; within Kanlow 0.07 to 0.29; within Summer 0.39 to 0.54.

A mapping population consisting of 100 progeny from a cross between the Kanlow and Summer genotypes with the highest dissimilarity score was produced during 2001. The parents and progeny population are maintained in the greenhouse at the UGA and will be used to construct a future map based on the polymorphic RFLP probes.

DISCUSSION

The lowland cultivars, Alamo and Kanlow, continued as the highest yielding switchgrass cultivars currently available for the southeastern United States, and support our original decision to base our cultivar improvement program on these germplasm sources. Although most summers during the past five years have been hot and dry, their annual dry matter yield continue to outperform the upland cultivars. These results confirm previous findings from Alabama (Maposse et al. 1995).

Our model for developing new switchgrass cultivars was as follows: (a) randomly selected genotypes (1000 or more) from good parental material (e.g., Alamo, Kanlow) are space planted into a honeycomb selection design (Fasoulas and Fasoula 1995) and harvested for dry matter yield during a two-year period; (b) the highest yielding genotypes from this honeycomb space planted trial (normally 45-50 genotypes) are clonally replicated, polycrossed, and their half sib progeny are advanced into the next cycle of honeycomb selection (see “e” below) while seed of the best seed yielding genotypes (normally 20-25 genotypes) are also advanced into multiple location, replicated, half-sib performance sward trials; (c) based on these half sib performance trials the best performing genotypes are identified and these elite plants (normally 8-12 genotypes) are polycrossed to composite the Syn 1 generation of an experimental cultivar; (d) these experimental cultivars are tested for performance at multiple locations within Georgia, and when possible, in other states; (e) simultaneously with step b above, the next cycle of honeycomb spaced plant selection is also established but this time based on families of the best genotypes (e.g., 20 genotypes or replicates from the best 45-50 families to again give an initial of approximately 1000 plants) and the steps proceed as shown above. Therefore, the program forms a continuum occurring in several phases in any one year (e.g., evaluation of cycle 2 space plants, harvesting half-sib progeny performance trials, polycrossing elite genotypes, evaluating newly composited experimental cultivars, and planting newly-established cycle 1 genotypes of the latest parent material).

The solid heritability and genetic gain estimates for dry matter yield in switchgrass indicated an ability to produce a higher yielding cultivar than Alamo for the southeast. Therefore, this cultivar development program was placed on a "fast track" in hopes of obtaining a better performing cultivar within our initial five-year contract period. The four experimental synthetics produced thus far, and now in performance tests, could provide this cultivar. Initial performance results have been very promising, demonstrating a 30% yield enhancement over Alamo and Kanlow. Future testing, including testing in other states, will be critical before a determination to release into the commercial seed trade.

When compared to Tifton 85 bermudagrass, Tifton 9 bahiagrass, and Merkron napiergrass, Alamo switchgrass was found to show poorer yields than Merkron and Tifton 85, but better yields than Tifton 9. The exceptional performance of Tifton 85 bermudagrass is extremely noteworthy because this hybrid bermudagrass is also a variety of choice for many commercial hay producers in the lower south and would give any producers a very good option to produce either biomass for a biofuels initiative or sell as hay on the open market. Finally, Merkron has consistently showed highest dry matter yields. However, there continues to be some winter damage each year on this species at the Athens location indicating its real potential lies mainly in the Gulf Coast region of the southeast. The excellent characteristic of Tifton 85 and Merkron should therefore be enough to initiate more basic research on them by DOE for their chemical ability as a biofuels crop for either ethanol or methane production.

Throughout the project, we continued to encounter problems when establishing switchgrass directly into non-fumigated soil. Even when plants emerge and establish, their productivity during the initial year is very poor. The reason for this is not readily apparent, but could be partially due to the very hot and dry summer experienced during the project period. However, preliminary experiments demonstrated a need for using a pre-plant and post-plant herbicide program, especially when using no-till methods, to give more productive stands. Management research to better establish switchgrass will need to continue in the future.

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2001 ANNUAL REPORT

Objective 1. Initiate a breeding program to improve existing switchgrass varieties for the southeast.

Three new experimental synthetics produced by this project, GA-991, GA-992, and GA-993, were established in small plot performance trials at the Tifton and Athens testing locations during 2000. First-year data were highly variable due to dry weather and concurrent establishment problems. However, during 2001, full season yields were obtained and showed the high yielding ability of these experimentals at both test locations when compared to the Alamo and Kanlow checks.

Entry	Yield (kg ha^{-1})		Average yield (kg ha^{-1})
	Athens	Tifton	
GA-993	12,563	20,791	17,500
GA-991	11,716	21,162	17,384
GA-992	10,540	20,976	16,801
Kanlow	9,755	15,380	13,130
Alamo	6,313	18,088	13,378
LSD ($P < 0.05$)	1,620	3,457	2,067

Another cycle of phenotypic selection using the honeycomb design also allowed new genotypes to be identified Alamo and Kanlow which were also polycrossed, progeny tested over years and locations, and the best ones intermated to form an additional synthetics, GA-001. New small plot yield trials were established at Athens and Tifton in the spring 2001 to test the performance of this new synthetic against GA-991, GA-992, and GA-993 and the checks Alamo and Kanlow. During the early establishment phase of this trial, an outbreak of an unknown disease occurred. This disease greatly damaged the Alamo control and the GA-991 experimental, but GA-992, GA-993, and GA-001 shown high resistance that resulted in good yields for these experimentals by the autumn harvest.

Entry	Autumn harvest ($kg\ ha^{-1}$)
GA-001	2060
GA-993	1990
GA-992	1415
GA-991	991
Alamo	558
LSD (P < 0.05)	904

The vast majority of the newly planted half-sib lines continue to out-yield both the Alamo and Kanlow checks at two test locations. The highest performing genotypes based on seed yield and dry matter yield performance across multiple locations will be identified for possible use in forming future experimental synthetic cultivars.

Objective 2. Provide data on yield performance of the best currently available switchgrass varieties and any new selections on representative soil types of the southeast.

The 2001 performance data from the long term experiments previously established at Tifton and Athens continue to show more yield for the lowland varieties, Alamo and Kanlow, at both testing locations than the upland varieties, Cave-in-Rock, Shawnee, NE Late, and Trailblazer. The drop off in performance of all the varieties, especially Kanlow, during 2000 at both locations was not repeated in 2001. High yields were the norm during 2001 probably due to higher rainfall.

Site and variety	Yield (kg ha^{-1})					
	1996	1997	1998	1999	2000	2001
Athens:						
Alamo	6,693	21,379	16,627	18,978	10,982	22,139
Kanlow	5,713	23,153	15,627	19,713	6,890	21,914
Cave-in-Rock	4,630	14,747	14,012	11,617	7,050	12,890
Shawnee	4,513	13,502	11,006	12,096	5,843	11,688
NE Late	4,368	13,276	12,742	11,617	6,513	14,122
Trailblazer	4,128	12,933	10,694	9,295	5,085	10,263
LSD (5%)	1,371	3,882	2,406	2,195	2,526	3,471
Tifton:						
Alamo	6,312	13,840	21,597	17,717	16,641	21,834
Kanlow	6,414	19,394	20,452	14,828	12,786	21,813
Cave-in-Rock	4,641	5,702	10,092	6,898	6,235	11,954
Shawnee	5,110	5,730	11,236	6,931	6,726	12,670
NE Late	4,609	4,152	8,976	5,265	4,364	9,354
Trailblazer	4,093	4,400	8,247	5,032	4,295	8,441
LSD (5%)	1,411	1,664	7,037	1,426	2,139	3,860

Objective 3. Evaluate the potential of selected alternate herbaceous species that may compete with switchgrass for herbaceous biofuels production in the southeast.

In all previous years, Merkron napiergrass produced very high dry matter yields compared to the other species. However, during 2001, the dry matter yield of napiergrass was greatly reduced compared to the other species and it actually produced less yield than switchgrass and bermudagrass at the Athens and Tifton testing locations. At the Midville testing location, napiergrass continued to be the highest yielding species, but again its yield was much lower than in previous years and not significantly different from bermudagrass.

Site and variety	Yield (kg ha^{-1})					
	1996	1997	1998	1999	2000	2001
Athens:						
Napiergrass	23,808	32,139	33,625	39,612	19,239	16,316
Bermudagrass	9,267	24,895	16,606	13,881	18,521	22,572
Switchgrass	6,693	21,379	16,627	18,978	10,982	22,139
Bahiagrass	2,168	18,404	11,694	11,235	7,454	16,444
Tifton:						
Napiergrass	41,628	30,821	39,778	17,930	24,917	13,344
Bermudagrass	9,856	15,840	24,364	15,466	19,214	20,407
Switchgrass	6,312	13,840	21,597	17,717	16,641	21,834
Bahiagrass	4,527	11,563	18,817	10,196	8,404	11,217
Midville:						
Napiergrass	--	20,111	48,886	45,913	20,107	18,571
Bermudagrass	--	11,820	24,179	22,086	12,505	17,211
Bahiagrass	--	9,508	17,283	14,005	7,461	10,395
Switchgrass	--	6,814	15,439	17,738	4,156	13,106
LSD (5%)	--	1,972	5,002	5,235	3,025	4,180

Objective 4. Develop a genetic framework map for switchgrass based on the single dose restriction fragment approach; develop a genetic map that includes RFLP and SSR markers.

All autogradiographs were scored for the presence or absence of bands. Data from the first 68 RFLP probes were analyzed by SYSTAT 10.1 using probes and enzymes as independent variables and genotypes as dependent variables. Hierarchical clustering was used to determine the distance matrix between the different genotypes. Eighty-seven percent of the probes used hybridized to the switchgrass DNA and 81% were polymorphic. Most of the polymorphism observed was between the cultivars. The dissimilarity between Kanlow and Summer ranged from 0.71 to 0.80; between
Summer and Alamo 0.64 to 0.85; within Alamo 0.03 to 0.33; within Kanlow 0.07 to 0.29; within Summer 0.39 to 0.54.

A mapping population consisting of 100 progeny from a cross between the Kanlow and Summer genotypes with the highest dissimilarity score was produced during 2001. The parents and progeny population are maintained in the greenhouse at the UGA and will be used to construct a map based on the polymorphic RFLP probes.

Activities

One Ph.D. graduate student, Ali Missaoui, continues to be supported by this project. His dissertation research is centered on general breeding and genetics research, including the use of molecular markers, with switchgrass.

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