

## PROGRESS REPORT \_\_\_\_\_ L

THE DEVELOPMENT OF NEW TECHNIQUES OF USING IRRADIATION IN THE GENETIC IMPROVEMENT OF WARM SEASON GRASSES, THE ASSESSMENT OF THEIR GENETIC AND CYTOGENETIC EFFECTS AND BIOMASS PRODUCTION FROM GRASS

By Glenn W. Burton, Research Geneticist  
and  
Wayne W. Hanna, Research Geneticist

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Approved for submission:

Glenn W. Burton  
Glenn W. Burton, Research Geneticist  
Co-Principal Investigator

6/18/79  
Date

E. Broadus Browne  
E. Broadus Browne, Director  
Georgia Coastal Plain Station

6/18/79  
Date

William O. Burke  
William O. Burke  
Executive Vice President  
University of Georgia  
Research Foundation, Inc.  
Phone: (404)542-3360  
(To receive notification of  
award and copies of correspondence)

6/25/79  
Date

MASTER

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PART I

DEVELOPMENT OF NEW TECHNIQUES OF USING IRRADIATION IN THE GENETIC IMPROVEMENT OF WARM SEASON GRASSES, THE ASSESSMENT OF THEIR GENETIC AND CYTOGENETIC EFFECTS AND BIOMASS PRODUCTION FROM GRASS.

INTRODUCTION

We believe that irradiation and chemical mutagens can be a very valuable tool to the plant breeder. Under this contract we hope to develop new techniques of using irradiation and chemical mutagens in the genetic improvement of several warm season grasses. We also hope to determine the economic value of radiation induced mutants. Genetic and cytogenetic effects of these treatments will also be studied.

Part A-1. Altering Protein Quantity and Quality in Pearl Millet Grain by Irradiation and Mutation Breeding.

In the spring and early summer of 1974, we planted in a field that had been uniformly cropped to velvet beans in 1973, 13,612 different pearl millet seed lots. These were remnants that had been accumulated as we carried out our study on general and specific combining ability under earlier contracts. A single 10-foot row of each remnant (for which enough seed was available) was planted on uniformly fertilized soil. In order to spread the work load, we planted 4062 plots of inbred Tift 239 on 5/6/74; 2926 plots of inbred Tift 23 on 6/17/74; 3240 plots of inbred Tift 13 on 5/27/74; and 3384 plots of inbred Tift 18 on 7/11/74. Tift 23 is the maintainer for A<sub>1</sub> sterile cytoplasm used in producing hybrid millet throughout the world. Tift 239 and Tift 13 are maintainers for A<sub>2</sub> cytoplasm that will be used to reduce the genetic vulnerability associated with the use of one sterile cytoplasm. Tift 18 is being recommended in West Pakistan as a variety and also gives a good fertility restored F<sub>1</sub> hybrid when crossed on Tift 23A<sub>1</sub>.

As the plants in these 13,612 plots headed, we covered at least 4 heads on each plot with special kraft bags to prevent cross pollination

and protect the seed from insect and bird damage. When mature, the heads were harvested, dried, and threshed.

Seed production on Tift 239 was very good but adverse weather conditions resulted in poor seed set on the other inbreds. Many heads that were threshed failed to have enough seed for chemical analyses. A total of 13,236 seed lots with enough seed for analysis and a remnant for planting were sent to Dr. R. Rabson, International Atomic Energy Agency, Karntner Ring 11, P.O. Box 590, A-1011 Vienna, Austria for protein and amino acid analysis in January and February, 1975. In the summer of 1975, we planted in the field short single row plots of a number of pearl millets to be checked for protein and lysine content. Included were 1130 lines out of Tift 239DB that had shown better than average protein and lysine content in analyses that were conducted on 1974 material. These were planted in the field with 3 replications. Also we produced seed on 1667 lines of inbred 18B and 2272 lines of inbred 23B. In the latter 2 cases, we grew out only one replication of each line. As these lines began to head, we covered at least 4 heads on each plot with special kraft bags to prevent cross-pollination and to protect the seed from insect and bird damage. When mature the heads were harvested, dried, and threshed. These seeds have been sent to Dr. R. Rabson, International Atomic Energy Agency in Vienna, Austria, where they were analyzed for protein and lysine content.

In 1976, we planted 1872 plots with seed sent by Dr. Rabson for replicated planting or remnants as yet not screened originally. Selfed seed was produced, threshed, and processed on these plots to ship to Vienna.

Dr. Rabson developed the first paper from this effort and pre-

sented it at the Research Coordination meeting in Vienna. The reference is: Rabson, R.; G. W. Burton; W. W. Hanna; H. Axmann; and B. Cross. The Development of Procedures for the Selection of Genotypes for Improved Protein of Pearl Millet from Mutagenized Populations of Inbreds, International Atomic Energy Agency, Vienna. 57/32:279-281, 1978. A copy of this paper was appended to our August, 1977 report.

Our replicated tests have yielded 9 and 1 lines from inbreds Tift 239DB and Tift 23B, respectively, with improved seed quality. The amino acid profile and N content of 3 selections from inbred 239DB and 1 from inbred 23B are summarized in Table 1. The data indicates that improved seed quality types are being recovered. The advanced selections were replicated again in 1978 to see if the improved quality characteristics are constant but analyses have not been received at the writing of this report.

Earlier tests with normal and translocated inbred 13B indicated higher levels of protein and lysine in the translocated line. In 1977, 32 translocated lines were grown and the seed analyzed for protein and dye-binding capacity. The crude protein of the t stocks is summarized in Table 2. The data show a range in crude protein of 10.6 to 18.1%. All of the chromosomes in the t stocks have not been identified, so definite conclusions cannot be drawn. However, for the t stocks that have been identified, the AB translocated chromosome stock tends to have higher protein. Similar variability has been observed in dye-binding capacity.

#### Part A-2. Effect of Nitrogen and Genotype (male and female) on Pearl Millet Grain

Environment and genotype have been shown to affect size and composition of grain in a number of cereals. In self-pollinated species such as wheat, oats, and sorghum, the male and female are the same

Table 1 . Amino acid composition of grain protein in pearl millet selections (Values expressed in g amino acid/16 g N).

	Inbred 23DB				Inbred 23B	
	Control <sup>a</sup>	13117 <sup>a</sup> (248)	6100 <sup>a</sup>	13129	Control	27
Lysine	3.59	3.13	3.44	4.10	2.79	3.60
Histidine	2.88	3.07	3.02	3.22	3.00	4.86
Arginine	5.89	6.20	7.31	7.23	5.29	7.70
Aspartic	10.16	10.78	11.27	12.47	10.88	10.89
Threonine	4.11	4.14	4.13	4.12	4.68	4.34
Serine	5.33	5.55	5.46	5.15	6.15	5.44
Glutamic	26.87	27.20	25.72	24.42	27.96	25.46
Proline	9.64	10.83	9.52	8.28	8.82	8.24
Glycine	3.69	3.75	4.43	4.69	4.57	4.41
Alanine	10.31	9.26	9.12	8.76	10.33	9.71
Half cystine	0.54	0.76	0.98	1.01	1.84	0.35
Valine	7.26	7.23	7.00	7.77	7.31	7.27
Methionine	3.69	3.54	3.04	2.96	2.15	2.20
Isoleucine	6.38	5.26	4.91	5.10	5.87	5.13
Leucine	14.08	13.36	12.29	11.61	13.58	12.79
Tyrosine	4.11	4.38	3.85	4.15	4.13	3.22
Phenylalanine	6.51	6.42	6.31	6.05	6.33	6.15
Recovered N % of sample	1.63	1.96	2.03	2.10	1.40	1.77

<sup>a</sup> Data represent average of two analyses

Table 2 . Percent crude protein in seeds of pearl millet translocation tester stocks.

t stock number	Chromosomes involved	% Crude protein
38		11.3
39		10.6
40		13.8
41		13.1
42		12.5
44		13.1
46		15.6
47		10.6
49		10.6
51		16.9
51		14.4
51		13.8
50		13.8
55		14.4
51		15.0
51		15.0
52		16.3
52		16.9
53		17.5
3	AB	17.5
3	AB	18.1
4	CD	16.3
6	CE	13.8
7	CG	15.6
7	CG	15.6
8	AC	14.4
9	CG	13.8
14	CD	13.8
15	AD	15.6
18	BE	14.4
18	BE	16.9
19		16.9
19		18.8
20		16.9
20		16.3
22		15.6
22		14.4
23		16.9
23		15.6
26		16.9
28		16.3

Table 2 (con't). Percent crude protein in seeds of pearl millet translocation tester stocks.

Stock number	Chromosomes involved	% Crude protein
29		16.9
32		16.3
33		16.3
33		13.8
34		14.4
34		14.4
23DB check		14.4
23DB check		16.3

$\bar{X}$  - 15.07  
Range - 10.6 - 18.1  
S.D. - 3.62

genetically and should have no differential effect on the grain. In cross pollinated species such as corn and pearl millet, half of the embryo germplasm and one-third of the endosperm germplasm (the male component) usually come from a different genotype. In 1952, we showed that the pollen source had an immediate gametic effect on seed size that ranged from 0 to 40% in 60  $S_3$  lines and increased seed size over selfing an average of 18.9% (Burton, Agron. J., 44:424-427). This experiment was designed to measure the male and female genotypic effects of six genotypes, Tift inbreds 13, 12 (13 with no tr) 18DB, 23DB, 239DB, and Bil 3B when fertilized with 15, 80, or 150 kg/ha of N. A split plot randomized block design with 4 reps was used with nitrogen being the whole plots; female genotype, the subplots; and male genotype, the sub subplots. The males consisted of pollen from the six inbreds (sibbing when pollen from a line was applied to itself), selfing and out-crossing with all millets to give the open pollinated seed. With a few exceptions, 576 heads were involved and covered with insecticide treated kraft bags to protect them from birds and insects. The seeds were harvested, threshed, cleaned, packaged, and sent to Dr. R. Rabson for chemical analyses. Mr. Ben Mullinix, Coastal Plain Station statistician used a special least squares analysis to analyze the data that contained quite a few missing values.

An analyses of the results of this study carried out in 1978 proved that nitrogen and the male and female significantly affect the seed characteristics measured in this study. Although the male gametic effect was smaller than the female gametic effect, it was large enough to suggest that selfed or sibbed seed of cross pollinated crops should be used in their genetic assesment of grain size and composition.

The results of this study have been prepared for publication in Crop Science under the title "Immediate effect of male and female gametes and nitrogen on five pearl millet grain characteristics". A copy of the manuscript presently in the hands of the editors appears with reprints in this report.

Part B. Irradiation Breeding of Sterile Triploid Turf Bermudagrasses.

Tifgreen, Tifway, and Tifdwarf are sterile interspecific triploid hybrids that cannot be improved by conventional breeding methods. From 1969 to 1972, we studied a number of techniques of creating mutations in these three hybrids and found that subjecting dormant rhizomes to gamma rays from a USDA Cobalt 60 source located on the Coastal Plain Station was the most effective method of creating mutations. From these efforts came 158 mutants that were obviously different from the parent material that was irradiated. Specialists and golf course superintendents observing these mutants that are not planted in the field have frequently observed, "sure they're interesting but are they really any better than Tifgreen, Tifway, and Tifdwarf". The only way that this question can be answered is to subject these mutants to a very thorough evaluation in plots that are managed as for golf greens and fairways.

The first set of these mutants of both Tifdwarf and Tifgreen were planted in 9 x 9 foot plots in June of 1970. The second set were similarly planted in the field on April 5, 1972. Ratings on color, vigor, growth habit, percent ground cover, and sod density have been made on these plots for a number of years.

In the fall of 1972, above ground stems that could not be infested with nematodes were harvested from each of these mutants and were planted in the greenhouse bed that had been artificially infested with

root knot nematode, Meloidogyne graminis by Dr. A. W. Johnson. Ten sprigs of each grass was planted and was allowed to grow until the root systems had developed. The plants were then carefully harvested, soil was washed from the roots and the roots were stained. Each root system was then carefully examined for the occurrence of stained nematodes in the roots. The results of these studies, presented in the 1972 annual report, indicated that none of the mutants showed a high level of resistance to root knot nematode but several mutants appeared to have greater resistance than the parent clonal material from which they arose. Purple anthocyanin content in the Tifgreen mutants was generally reduced below the parent material but mutants also occurred that had greater concentration of this purple plant color. Tifway bermudagrass treated exactly as Tifgreen yielded only about half as many mutants. Striking variation in rate of spread, morphology, etc. were observed. Our 1974 report included tables recording the variations that had been observed in field readings taken on these bermudagrass mutants. Ratings made on these plots in 1975 through May 1976 were summarized and recorded in our 1975 report. These ratings indicated that some mutants that had shown promise earlier no longer do but there are still mutants that appear to be equal or perhaps superior to their parental clones.

Our 1975 report also summarized research by Dr. A. W. Johnson on the nematode resistance of some of the best mutants. He found two mutants, Tifgreen 72-59 and Tifway 114 on which root knot nematodes were unable to reproduce themselves in his greenhouse test. Two other mutants, Tifway 117 and Tifdwarf 45 were not adversely affected by the rather heavy population of nematodes they carried.

In the winter of 1976-77, the parent clones, Tifway, Tifdwarf, and Tifgreen and 9 of their best mutants were increased vegetatively in 2-inch pots in the greenhouse. In April, 1977, they were set in the field in large (24 x 30 foot) plots in triplicate for evaluation under different managements. The soil was fumigated with methyl bromide for this experiment but the treatment was not well done and contamination of the test area with other bermudagrass and nut sedge had slowed the progress of this test.

In the summer of 1977, it was necessary to fumigate and replant several plots that were badly contaminated. Nutgrass in several other plots was sprayed with basagram, a systemic herbicide specific for nutgrass control. Basagram killed most of the aboveground nutgrass growth without injuring the bermudagrass. However, late in the season more nutgrass appeared demonstrating the need for repeated spraying with basagram for complete nutgrass control.

All plots became well established in 1978. Cutting height variables were initiated in 1978 and variable responses were noted.

Plugs from these plots and 22 other mutants and hybrids were planted on a golf course in Atlanta in 1978. They were also planted in small plots on the University of Georgia Turf Experimental Grounds as a part of their evaluation.

Part C. Irradiation Breeding of Sterile Coastcross-1, a Forage Grass Hybrid to Increase Winterhardiness.

Coastcross-1 is an outstanding sterile forage hybrid bermudagrass produced at the Coastal Plain Station that has far surpassed Coastal bermudagrass or other grasses in quality. However, Coastcross-1 lacks winterhardiness and since it is sterile, it cannot be improved by conventional breeding methods. We wish to determine if by

irradiation breeding it will be possible to increase the winterhardiness, perhaps destroy the dominant genes for non-rhizomatous condition and create a mutant that would have rhizomes.

Radiation breeding of Coastcross-1 was begun June 21, 1971. The main objective was to increase winterhardiness by restoring rhizomes or developing more winterhardy mutants. Some 400,000 freshly cut green stems of Coastcross-1 were packed into 14 x 16 x 36 inch bales with a standard hay baler. These were trucked to the University of Tennessee A.E.C. Agricultural Laboratory at Oak Ridge, Tennessee where they were exposed to 7,000 R. They were then trucked to the Mountain Experiment Station where they were broadcast and disked into the soil and were sprayed with 2,4-D to control weeds. Good establishment resulted and 25 chlorophyll deficient stem sectors were observed in the fall. Four tiny plants from irradiated material survived  $-16^{\circ}\text{C}$ . All plants from non-irradiated stems winterkilled. The four surviving plants are still being treated in replicated plantings for winterhardiness. Unfortunately, we have had unusually mild winters at the Mountain Experiment Station at Blairsville for the past four years and have been unable to get good readings on the winterhardiness of these four surviving plants.

We did observe on one of these plants a rhizome that was over 12 inches long, a much longer rhizome than we have ever observed before in non-irradiated material. We broke up this rhizome and planted it out in the field, hoping that it would give rise to plants that would be more rhizomatous than Coastcross-1. Observations in 1974 suggested that this material is no more rhizomatous than the parent clone. This material was used to establish plots in a new yield trial where it can be compared with the parent clone in yield and other important characters such as winter survival, spring growth, yield, etc. Unusually

mild winters at Blairsville in 1973-74 and 1974-75 permitted excellent recovery each spring of the irradiated Coastcross-1 and since there was no land available for planting, we did not treat and plant additional sprigs in the spring of 1974 or 1975.

The winter of 1975-76 was somewhat colder than usual and killed most of the Coastcross-1 plants on the field that had a north exposure (that sloped to the north). The field that sloped to the south lost comparatively few Coastcross-1 sprigs. On May 7, 1976, I carefully examined this field and concluded that there were too many sprigs surviving to permit one to conclude that they were, in fact, winterhardy. I dug up a number of plants and found that they had been able to establish shoots several inches below the surface of the ground from which the plants had been regenerated. Because of this deep placement of the shoots, it is not surprising that they survived the winter. I found one plant with a few rhizomes about 4 inches long and several others that had somewhat shorter rhizomes. I dug these up and brought them back to Tifton where we can study rhizome development more carefully. None of the plants demonstrated the rhizome development that we believe will be necessary if we are to have a Coastcross-1 that will perform dependably in colder climates.

Coastcross-1 is a tetraploid. For this reason, we might expect at least 2 dominant alleles for reduced rhizome development. If this is true, it is reasonable to suppose that the 4 tiny plants that survived the  $-16^{\circ}\text{C}$  in the winter of 1971-72 may have lost one of their dominant non-rhizomatous genes but not both of them. If this is true, we should be irradiating material from the best of these rather than from the original Coastcross-1. To test this theory and hopefully produce a rhizomatous Coastcross-1, we harvested at 6 a.m. on June 20, 1977, some 1.5 million sprigs from our increase of the best of the 4 tiny plants

that survived in 1971-72. These sprigs were baled with a regular hay baler, trucked to Oak Ridge and were put in the room for exposure to irradiation at 8:30 a.m. They were exposed for 5 3/4 hours to give about 7,000 r. They were then loaded on the truck and taken to Blairsville where they were broadcast, disked in, and sprayed with 2,4-D for weed control.

The 1977 planting became well established in the summer and the presence of chlorophyll deficient mutants proved that the irradiation exposure had been effective. However, none of the plants survived the severe 1977-78 winter.

Our plans to irradiate and plant more sprigs of the Coastcross-1 mutant could not be carried out because the land at Blairsville was too dry to plant during the few days that the irradiation source was available at Oak Ridge, Tennessee.

During 1978, we prepared for publication in the Journal of Environmental and Experimental Botany, the results of an evaluation study that proved that the best of the 4 irradiated plants of Coastcross-1 that survived the  $-16^{\circ}\text{C}$  in the winter of 1971-72 at Blairsville, Georgia is slightly more winterhardy than the original Coastcross-1. The manuscript entitled "An Induced Mutant of Coastcross-1 Bermudagrass with Improved Winterhardiness" is presently in the hands of the USDA editors. A copy of the manuscript appears with the reprints in this report.

Part D. Heterosis Resulting from Crossing Specific Irradiation Induced Mutants with their Normal Inbred Parent

Dr. C. O. Gardner at the University of Nebraska reported at the mutation breeding conference at Knoxville, Tennessee in February of 1972, a substantial amount of heterosis in a corn hybrid that had as one

of its parents a line carrying a noticeable specific chlorophyll mutant. From our pearl millet irradiation program have come a number of mutants that have specific characteristics that set them aside from the normal type on a morphological basis. Beginning in the summer of 1972, we hybridized a number of these mutants with the normal inbred line from which they came. Yield trials of such hybrids have been conducted from 1973 through 1978. Yield data collected through 1977 was summarized in the 1977 report and will not be repeated here.

In 1977, one 9 x 9 lattice square yield trial was devoted to a study of the yield performance of  $F_1$  hybrids between Tift 239DA and specific mutants of Tift 239DB. The CV of 8.20 for the total 1977 production indicates that the test was precise and the results were dependable. None of the mutants crossed with Tift 239DA yielded significantly more than Tift 239DA x normal Tift 239DB. Thirteen yielded significantly less at the 5% level. Hybrids between 239 and 23DB (dwarfs) yielded 34 to 52% more than the check, 239DA x 239DB.

In 1978, one 9 x 9 lattice square yield trial was again devoted to this test. Less favorable growing conditions and lower mean yields raised the CV for total yield for this test to 12.2% (See Table 3). The yield data presented in Table 1 shows that none of the Tift 239DB mutants crossed on Tift 239DA significantly increased its yield. Three mutants produced hybrids with Tift 239A that yielded significantly less than the check Tift 239DA x Tift 239DB. One of these three hybrids was not tested in 1977 but only one of the remaining two was in the group of 13 that yielded less than the check in 1977.

In 1978, 16  $F_1$  hybrids between Tift 23DA and Tift 239DB mutants were included in the 9 x 9 yield trial (Table 3). These hybrids yielded 51 to 70% more than the check Tift 239DA x Tift 239DB. Two of these yielded significantly more than the lowest, Tift 23DA x 7809 if the

Table 3. 1978 yield performance of hybrids involving mutants of pearl millet inbred Tift 239B.

Line or Hybrid	Pounds dry matter/acre when cut:			
	7/26/78	8/30/78	10/17/78	Total
76-239DA x 5935	1,959	2,379	903	5,254
77-239DA x 7748	2,915	2,647	1,129	6,674
76-239DA x 5936	2,516	2,468	914	5,873
76-239DA x 5938	3,736	2,627	1,034	7,389
76-239DA x 5945	1,843	2,417	728	5,028
77-239DA x 7750	3,384	2,626	1,053	7,067
76-239DA x 5955	3,742	2,666	1,002	7,413
76-239DA x 5941	2,496	2,691	965	6,112
77-239DA x 7754	2,771	2,452	1,013	6,237
76-239DA x 5957	3,104	2,374	1,141	6,622
76-239DA x 5958	2,593	2,477	809	5,881
77-239DA x 7787	2,184	2,749	1,010	5,989
77-239DA x 7789	3,085	2,632	1,099	6,812
76-239DA x 6003	3,532	3,022	1,129	7,658
76-239DA x 6023	3,208	2,315	889	6,422
76-239DA x 6025	3,051	2,512	1,077	6,663
77-239DA x 7794	2,607	2,619	947	6,218
77-239DA x 7794	3,388	2,835	1,080	7,273
77-239DA x 7802	3,047	2,423	964	6,421
77-239DA x 7803	3,480	2,620	952	7,075
77-239DA x 7804	2,919	2,700	1,079	6,744
76-23DA x 6034	2,494	2,668	934	6,092
76-23DA x 6037	1,926	2,616	1,046	5,529
76-239DA x 6040	2,994	2,355	825	6,171
76-239DA x 6042	3,351	2,622	1,020	6,988
76-239DA x 6043	3,306	2,720	1,136	7,144
76-239DA x 6044	2,960	2,657	988	6,557
76-239DA x 6045	2,268	2,578	989	5,867
76-239DA x 6047	2,912	2,624	1,060	6,573
76-239DA x 6050	2,589	2,394	1,038	6,047
76-239DA x 6059	3,114	2,319	1,070	6,522
76-239DA x 6060	3,132	2,860	1,104	7,060
77-239DA x 7819	3,832	2,327	1,026	7,234
76-239DA x 6094	4,033	2,866	947	7,774
77-239DA x 7827	3,170	2,373	927	6,484
76-239DA x 6114	2,669	2,236	992	5,840
76-239DA x 6139	3,350	2,171	791	6,308
76-239DA x 6147	2,254	2,821	1,291	6,397
76-239DA x 6157	3,727	2,751	925	7,391
77-239DA x 7859	3,333	2,443	1,008	6,747
76-239DA x 6158	3,032	2,674	1,003	6,702
76-239DA x 6159	2,979	2,347	898	6,289
76-239DA x 6164	2,463	2,483	740	5,726
76-239DA x 6165	2,858	2,890	1,057	5,812
76-239DA x 6183	3,761	2,161	863	6,769
76-239DA x 6195	2,477	2,398	941	5,853
76-239DA x 6225	3,746	2,650	1,078	7,480

Table 3 (con't). 1978 yield performance of hybrids involving mutants of pearl millet inbred Tift 239B.

Line or Hybrid	Pounds dry matter/acre when cut:			
	7/26/78	8/30/78	10/17/78	Total
76-239DA x 6231	2,178	2,940	1,084	6,198
76-239DA x 6289	2,569	2,510	860	5,879
77-239DA x 7884	3,684	2,831	1,059	7,579
76-239DA x 6266	2,922	2,543	909	6,404
77-239DA x 7872	2,034	2,695	1,011	5,753
76-239DA x 6300	3,372	2,475	1,059	6,942
77-239DA x 7887	3,791	2,488	1,053	7,265
77-239DA x 7890	2,510	2,803	1,006	6,280
77-239DA x 7891	2,940	2,379	933	6,264
77-239DA x 7893	3,445	2,380	967	6,797
77-239DA x 7894	3,275	2,784	1,035	7,074
77-239DA x 7896	2,679	2,756	1,074	7,467
77-239DA x 7898	3,723	2,419	914	7,106
77-239DA x 7900	3,343	2,849	935	7,179
77-239DA x 7920	3,512	2,377	854	6,748
77-239DA x 7903	2,557	2,577	905	5,979
77-239DA x 7905	3,170	2,821	975	6,987
77-23DA x 7748	6,378	3,757	1,401	11,513
77-23DA x 7750	5,857	3,820	1,293	10,908
77-23DA x 7751	6,397	3,775	1,476	11,636
77-23DA x 7789	5,346	3,620	1,361	10,359
77-23DA x 7794	5,368	4,009	1,389	10,710
77-23DA x 7797	5,327	3,585	1,626	10,534
77-23DA x 7802	5,676	3,414	1,341	10,431
77-23DA x 7803	5,131	3,823	1,442	10,377
77-23DA x 7804	5,412	4,093	1,523	11,023
77-23DA x 7805	5,194	4,133	1,533	10,858
77-23DA x 7807	6,077	3,608	1,376	11,102
77-23DA x 7806	5,078	3,616	1,414	10,112
77-23DA x 7808	5,519	3,656	1,445	10,612
77-23DA x 7809	5,324	3,675	1,254	10,248
77-23DA x 7812	5,948	3,739	1,309	11,020
77-23DA x 7817	6,028	3,730	1,230	11,045
239DA x 239DB	3,110	2,585	1,052	6,784
C.V.	22.15	12.10	16.00	12.21
5% LSD	976.29	425.22	214.26	1130.82

5% LSD of 1131 is used as a "measuring stick" for significance. However, this LSD is an average value for all entries in the test and is too low for these hybrids and too low for the Tift 239A x mutant hybrids.

The results of this 2-year evaluation of the yield of  $F_1$  hybrids involving specific mutants of Tift 239DB indicate that none of these mutants were able to increase forage yields.

#### Part E. Economic Assessment of Irradiation Induced Mutants

No one doubts the ability of irradiation to create variation in plants. This has been well established. A great many plant breeders, however, question whether any of these mutants have economic significance. Certainly a large proportion of them do not. We propose to test those that appear to be most promising in an effort to ascertain whether or not mutants of economic importance can be developed from irradiation breeding and what mutation techniques are most likely to produce them.

A mutation with potential for improving quality of forage as well as pest resistance is the bloomless mutation (removes wax from the plant) in sorghum. Since 1975, progress has been made toward developing a bloomless sudangrass and sorghum-sudangrass hybrid that should be more digestible and give better daily gains when grazed by cattle. In 1978, seeds of bloom and newly developed bloomless Georgia 337 were increased for grazing trials in 1979.

#### Part F-1. Use of Ethidium Bromide to Create Cytoplasmic Male Sterile Mutants in Pearl Millet

The observation (confirmed with more research over the past several years) that the cytoplasmic male sterile lines Tift 23DA and Tift 23A could mutate to pollen shedding maintainers was proof of mutation in the cytoplasm. It occurred to us, therefore, that if we

could find mutagenic agents specific for the cytoplasm, it might be possible to treat the pollen shedding maintainer and create cytoplasmic male steriles. I discussed this idea with those attending the FAO/International Atomic Energy Agency sponsored meeting in Japan in the fall of 1974. They suggested that ethidium bromide had been effective in creating mutants in the cytoplasm of yeast and that Dr. Ashri in Israel had used this mutagenic agent on peanuts. I contacted Dr. Ashri obtained the benefit of his experience and in the summer of 1975, carried out an experiment on the use of ethidium bromide to create cytoplasmic male steriles in the inbred lines of Tift 23DB. The experiment proved so successful and the follow-up work in the greenhouse so encouraging that we prepared a brief article for publication in Crop Science. The reprint of this paper was attached to the 1977 report.

In the summer of 1976, we grew out progenies ranging from 3 to more than 50 plants, from plants in the greenhouse that we had classified as probable cms plants. They had been pollinated from pollen shedding sister plants or in some few instances, with pollen from Tift 23DB. The number of cytoplasmic male sterile and male fertile plants in each progeny were determined and recorded. These varied from none to a little more than half of the progeny in which they occurred. Since most of the plants were shedding pollen, the male sterile plants set seed. A few controlled crosses were made on sterile plants using 23DB pollen. None of the cms lines arising from ethidium bromide seed treatment were 100% male sterile. Several lines produced a low frequency of pollen shedding plants suggesting that the frequency of pollen shedding plants can be reduced by selection. Cms plants in the best lines were pollinated with 23DB pollen in the field in the summer of 1977. These were advanced another generation in the greenhouse and were taken to the field for assessment in the summer of

1978. Careful examination of pollen shed and seed set under bag of several thousand plants has forced us to conclude that the frequency of pollen shedders in our ethidium bromide mutants cannot be significantly reduced by selection.

Part F-2. Use of Mitomycin and Streptomycin to Create Cytoplasmic Male Sterile Mutants in Pearl Millet.

In the summer of 1977, we grew at Tifton about 70,000 plants from Tift 23DB seed that had been soaked in water solutions of mitomycin and streptomycin for 40 hours at 5°C. The concentrations used were: 50 ppm of mitomycin and 200 ppm and 500 ppm of streptomycin. Treated seeds were surface-dried and planted in the field immediately. Alternate equal sized strips of the treated and a check were planted. The check produced some 32,800 plants whereas the 3 antibiotics mitomycin - 50 ppm, streptomycin - 200 ppm and streptomycin - 500 ppm reduced seed viability to give only 13,000, 22,300, and 18,000 plants, respectively, (Table 1). The frequencies of mutants observed in this M<sub>1</sub> material in 1977 are recorded in Table 4.

On July 20, 1978, M<sub>2</sub> progenies of more than 50 plants were grown from open pollinated seed of each M<sub>1</sub> mutant observed in 1977. Since these M<sub>1</sub> mutants occurred in a field of Tift 23DB with all plants except the mutants shedding pollen, open pollinated seed would be in effect Tift 23DA mutant maintained with Tift 23DB pollen. Table 1 shows that only one-third to one-fourth of the M<sub>1</sub> mutants produced progenies with some partially male sterile plants.

Of particular significance is the number of progenies in all treatments that were as completely cytoplasm male sterile at Tift 23DA. The occurrence of 2 of these in the check material was unexpected. A similar number of "check" heads from untreated seed of the same seed

Table 4.. Effect of mitomycin and streptomycin seed treatments on cytoplasmic male sterile mutant frequency in Tift 23DB.

	Check	Mito- mycin 50 ppm	Streptomycin	
			200 ppm	500 ppm
<u>Seed planted 7/6/77</u>				
M <sub>1</sub> heads produced	32,800	13,000	22,300	18,000
M <sub>1</sub> partially male sterile head mutants	13	24	9	22
M <sub>1</sub> whole head male sterile mutants	20	36	21	9
<u>Seed planted 7/20/78</u>				
M <sub>2</sub> progenies with some partially male sterile plants	8	21	10	11
M <sub>2</sub> progenies with all plants cytoplasmic male sterile	2	4	4	4
M <sub>2</sub> completely cytoplasmic male sterile progenies per 16,000 M <sub>1</sub> heads	1	4.9	2.9	3.6

lot of Tift 23DB examined in 1975 (when the ethidium bromide mutants were created) contained no cms mutants. Two explanations are suggested. Either there is a spontaneous mutation frequency of one cms head per 16,000 or there was a mixture of one cms Tift 23DA seed per 16,000 Tift 23DB seeds in the seed lot treated in both studies. In either case data in Table 4 show that the mutagenic agents increased the mutation rate for cms in Tift 23DB from 2.9 to 4.9 times.

In 1978 many of the cms plants on each row were crossed with different maintainers and fertility restorers in an effort to ascertain if they are different than Tift 23DA. These will be grown out in the summer of 1979.

#### Part G. Biomass of Napiergrass.

Napiergrass or elephant grass, Pennisetum purpureum is a giant perennial grass that originated in Africa. Reports from Hawaii and Puerto Rico indicate that it can outyield all other grasses including sugar cane. The national energy concern and the need to assess biomass production as a partial solution to the energy needs in the future motivated the following study initiated in 1978.

The variety chosen for this study was Merkeron, an  $F_1$  hybrid we produced at Tifton in the 1940's. This hybrid outyielded others tested in Puerto Rico in the 1940's and has been used as a greenchop forage grass for milk cows in the tropics. It has survived winters at Tifton of  $-14^{\circ}\text{C}$  but its northern limits are not known.

In the spring of 1978, stem cuttings were planted in the greenhouse in 10 cm pots and these rooted single stems with plants 15-20 cm tall were set in the field on .9 x .9 m centers on May 5. The fertilizer treatments described in Table 5 were applied to 3-row plots 5.6 m long in triplicate on May 23 and June 23, 1978. The grass grew well through-

Table 5. 1978 biomass production of Merkeron napiergrass set as rooted single stem cuttings on .9 m centers 5/5/78 and cut 11/21/78.

Treatment	Fertilizer applied kg/ha of			Dry matter yield Kg/ha	Chemical content			IVDMD %
	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O		Protein %	P %	K %	
1	120	60	120	38.7	6.4	.12	0.96	41.2
2	360	180	360	35.1	6.5	.09	0.78	35.9
3	480	240	480	39.0	6.7	.12	1.13	48.7
4	480	240	480	50.7	7.6	.12	1.24	46.1

Note: All of fertilizer treatment 1 and half of 2, 3, and 4 were applied 5/23/78.  
The other half of 2, 3, and 4 was applied 6/23/78.

Napier was 4 m tall when cut with a standard John Deere forage harvester.  
The forage contained an average of 26.3% dry matter when harvested.

out the summer with no insect or disease attacks.

On November 21, when the test was harvested with a standard John Deere forage chopper, the napiergrass was 4 m tall showing no signs of lodging. The lower leaves on the inside rows were dead but were still attached to the stems and could be harvested without loss. The yields reported in Table 5 were taken from the middle row in each 3-row plot. These had been planted to more vigorous rooted cuttings and outyielded the other two rows. If the yields of the outside row of plants were discarded, the dry matter yield of the remainder of the test area would be 28.5 MT/ha.

Fertilizer treatments had no significant effect on any variable measured (Table 5). The results suggest that napiergrass allowed to grow all season can maximize its biomass production if it contains about 1% of N and K and 0.12% of P. The IVDMD of the dried ground forage ranged from 35.9 to 48.7 and was higher than expected.

Being a perennial, Merkeron napiergrass in this study should start growth much earlier in 1979 and should give a much higher total yield of biomass than that recorded in 1978.

#### Part H. Evaluation of Mutagen Induced Lignin Mutants in Sorghum.

Quality (digestible dry matter or energy) is as important in forage as dry matter yield. The diethyl sulfate induced brown midrib (bmr) mutants which significantly reduce lignin and increase in vitro dry matter digestibility (IVDMD) in grain sorghum (Crop Science 18:205-208, 1978, the only published report on the mutants) hold exciting potential for the improvement of forage sorghum.

Four near-isogenic brown midrib (bmr 6, 14, 17, and 18) and normal lines discovered in the Purdue grain sorghum breeding program were obtained in 1977 from Dr. Lynn Gourley at Mississippi State University.

The bmr 12 line was obtained from Kay Porter, Purdue University in 1975.

We were interested in the mutants for their forage potential and wanted to know at what stage of growth did the bmr genes express themselves. Therefore on May 19, 1978 we planted three replications (three plots per replication) of each mutant and its near isogenic counterpart arranged in a randomized complete block design. The test received 275 kg/ha 5-10-15. We harvested plants one month later on June 19 and then at two-week intervals until seed maturity.

The IVDMD data is summarized in Table 6. The data shows that all mutants except bmr 17 already had higher quality forage one month after planting. This is valuable information since most grazed sorghum would be utilized at this age and stage of growth.

The data on dry matter percent (Table 7) showed in general non-significant ( $P = .05$ ) differences between normal and bmr pairs. The one exception was bmr 18 which was slightly lower than its normal counterpart.

Total sugar data are summarized in Table 8. The data shows that in general the bmr lines are significantly ( $P = .05$ ) higher in total sugars than the normal lines. The one exception is bmr 12 where the normal line is significantly higher. We have no explanation for this other than bmr 12 is a taller grain type with forage potential whereas the other lines are 3- and 4-dwarf grain types. This data indicates that sugars apparently have no effect on IVDMD.

In 1978, we increased and produced over 150 kg of each bmr 12 and its normal counterpart since it has forage potential and is one of the highest IVDMD mutants. The seed will be used in 1979 to grow plants for silage. The silage will be fed to dairy animals in order to see if the increased IVDMD is realized in animal performance. This work will be done in cooperation with Dr. Joe Johnson in Dairy Science.

Table 6 . Percent IVDMD of normal (Bmr) and brown midrib (bmr) sorghum forage in 1978.

	% IVDMD when cut on:					ANOVA			SE	CV
	6/19	7/05	7/21	8/07	$\bar{x}$	Mutant	Date	M x D		
† bmr 6	78.8	77.2	73.7	70.3	75.0	*	**	*	2.10	2.85
Bmr 6	76.8	72.8	70.6	69.9	72.5					
$\bar{x}$	77.8	75.0	72.2	70.1	73.8					
bmr 18	82.5	79.6	77.3	77.3	79.2	**	**	NS	1.84	2.44
Bmr 18	77.4	72.0	69.8	66.5	71.4					
$\bar{x}$	80.0	75.8	73.6	71.9	75.3					
bmr 14	84.2	79.0	76.7	77.6	79.4	**	**	NS	3.91	5.23
Bmr 14	78.0	71.4	68.3	63.3	70.3					
$\bar{x}$	81.1	75.2	72.5	70.5	74.8					
bmr 17	76.9	73.8	79.3	71.0	75.3	**	*	**	3.29	4.53
Bmr 17	77.4	70.6	64.0	69.7	70.4					
$\bar{x}$	77.1	72.2	71.7	70.4	72.8					
bmr 12	81.7	79.0	78.8	76.2	78.9	**	**	NS	2.35	3.10
Bmr 12	76.4	74.6	69.3	69.5	72.4					
$\bar{x}$	79.1	76.8	74.1	72.9	75.7					

† lower case letters indicate mutant line

\*, \*\* Significant at P = .05 and .01%, respectively

	ANOVA				
Inbred	*	**	NS	NS	**
Mutant	**	**	**	**	**
I x M	*	**	**	**	**
SE	2.07	1.42	3.04	3.04	1.52
CV	2.63	1.89	4.22	4.22	2.05

Table 7. Percent dry matter in normal (Bmr) and brown midrib (bmr) sorghum forage in 1978.

	% dry matter when cut on:					ANOVA			SE	CV
	6/19	7/05	7/21	8/18	$\bar{x}$	Mutant	Date	M x D		
<sup>+</sup> bmr 6	11	16	15	15	14	NS	*	NS	1.66	11.08
Bmr 6	15	17	14	14	15					
$\bar{x}$	13	17	15	15	15					
bmr 18	10	18	15	15	14	*	**	NS	1.88	12.55
Bmr 18	12	21	16	16	16					
$\bar{x}$	11	19	15	15	15					
bmr 14	11	21	18	18	17	NS	**	NS	2.51	15.71
Bmr 14	10	18	17	17	15					
$\bar{x}$	14	20	15	15	16					
bmr 17	14	20	17	17	17	NS	NS	NS	5.76	33.94
Bmr 17	9	16	22	22	17					
$\bar{x}$	11	20	17	17	17					
bmr 12	13	17	17	17	16	NS	**	NS	1.94	11.44
Bmr 12	13	19	19	19	17					
$\bar{x}$	11	18	20	20	17					

<sup>+</sup> lower case letters indicate mutant line  
 \*, \*\* Significant at P = .05 and .01, respectively

	ANOVA			
Inbred	NS	NS	NS	NS
Mutant	NS	NS	NS	NS
I x M	NS	NS	NS	NS
SE	2.35	3.22	3.69	2.39
CV	19.61	17.88	21.72	14.94

Table 8 . Percent total sugar in isogenic normal and brown midrib sorghum

Isogenic lines	6/19/78	7/5/78	7/21/78	8/7/78	$\bar{x}$
† bmr 6	10.2	19.7	18.8	29.4	19.5
Bmr 6	7.1	14.8	17.4	20.5	14.9
$\bar{x}$	8.6	17.3	18.1	24.9	17.2
bmr 18	8.1	13.7	18.2	25.9	16.5
Bmr 18	8.6	10.5	15.0	22.5	14.2
$\bar{x}$	8.4	12.1	16.6	24.2	15.3
bmr 4	10.1	19.1	28.0	31.1	22.1
Bmr 4	9.7	13.8	19.2	23.7	16.6
$\bar{x}$	9.9	16.5	23.6	27.4	19.4
bmr 17	8.4	14.6	22.5	29.2	18.7
Bmr 17	7.2	11.4	19.6	28.3	16.6
$\bar{x}$	7.8	13.0	21.1	28.8	17.7
bmr 12	10.5	13.0	22.9	31.1	19.4
Bmr 12	12.6	17.7	26.0	33.6	22.5
	11.6	15.3	24.5	32.3	20.9
<u>5% LSD</u>					
Inbreds	2.2	1.9	3.2	3.4	1.5
Isogenic lines	NS	1.2	2.0	2.1	1.0
I x I	NS	2.7	4.5	4.8	2.1
SE	1.9	1.6	2.6	2.8	1.3
CV	19.9	10.8	12.5	10.1	6.9

† lower case letters indicate mutant line  
 \*, \*\* Significant at P = .05 and .01, respectively

Since 1975, the bmr 12 gene is also being backcrossed to B lines to produce a bmr cytoplasmic-genic male sterile. It is also being backcrossed to sudangrass to produce a bmr sudangrass. The ultimate objective is to produce a high quality bmr sorghum-sudangrass hybrid if the animal data in 1979 shows an advantage for the mutagen induced bmr gene.

#### Part I. Interspecific Transfer of Germplasm Using Gamma Radiation.

Interspecific hybrids are usually highly male and female sterile. Although they are of value for determining species relationships, the highly sterile hybrids are usually difficult to use in a backcrossing program to transfer germplasm between species. The transfer of germplasm between plant species through normal genetic recombination is possible only if some homology exists between genomes which allows for chromosome pairing and crossing-over to take place.

Napiergrass, Pennisetum purpureum, is a robust tetraploid (4x) perennial tropical grass that is highly resistant to many serious pests such as downy mildew (estimated to affect 20-25% of the 50 million acres for food), rust (a serious problem in the U.S. as well as abroad), ergot, leaf spots, insects and other pests of diploid (2x) pearl millet, Pennisetum americanum. Napiergrass contains both an A and B genome whereas pearl millet contains only the A genome. The pest resistance is carried on the B genome of napiergrass and there is no homology between the A and B genomes. The triploid (AAB) 21 chromosome interspecific hybrid obtained by crossing pearl millet (AA) x napiergrass (AABB) is easy to produce but is highly male and female sterile. In 1978 we were successful in doubling the chromosome number of the interspecific triploid. The hexaploid (42 chromosomes) is fertile (sheds pollen and sets seed). Gildenhuis and Brix (*Agroplanta* 1:77-84, 1969) found that they could not transfer desired traits of napiergrass to pearl millet with three back-

crosses of the hexaploid to pearl millet because there was elimination of the B genome. For this reason we have irradiated 6x pollen with 1, 2, 3, and 4 Kr of gamma radiation to produce translocations between the A and B genomes. The irradiated pollen was used to pollinate cytoplasmic-genic male sterile 'Tift 23DA'. Therefore, seeds produced are of hybrid origin. Over 500 seeds have been produced by this procedure in 1978. These will be planted in 1979. Plants with translocated chromosomes should be semi-sterile. These will be selfed to produce plants homozygous for the translocation which should be fertile. The plants will be rated for desirable agronomic characteristics such as rust resistance.

Part J. Production of Homozygous Translocation Tester Stocks.

Pearl millet is an extremely important world forage and food crop, yet information on the chromosomal location of genes and on linkage analyses is nonexistent. This type of information would be very helpful in a breeding and improvement program.

In 1972 we initiated the development of homozygous translocations in dwarf Tift 23B which will allow us to conduct our genetic analyses in a dwarf tester stock series with a uniform genetic background. We decided to irradiate pollen because this method would eliminate sectoring in the  $R_1$  generation and would also eliminate many of the aberrations that would be of no value to us. After testing a number of gamma radiation doses, we decided that 3 Kr would give us the best seed set and the highest frequency of reciprocal translocations as reported in Crop Science, 1974, Vol. 14, pages 600-601. Although reciprocal translocations are our main objective, we will be on the lookout for duplications, deletions, and other aberrations that would

be useful in studying gene action.

Each year selfed progenies of semi-sterile  $R_1$  plants are grown in the field. Fertile plants in these progenies are testcrossed onto normal plant types without a translocation. When a male parent of a testcross is homozygous for a translocation, all testcross progeny are semi-sterile. Since 1974, over 50 homozygous translocations have been isolated. Many of these have been intercrossed and cytologically examined to identify the broken chromosomes. In 1977, we were able to develop and identify our first set of seven tester stocks (T3, 4, 6, 8, 10, 11, 18) that represent two breaks (each in separate tester stocks) in each of the seven chromosomes (except chromosome F) of pearl millet. This tester set will simplify the identity of the remainder of the translocations and will allow us to begin linkage and gene action studies.

In 1978, forty-seven (47) additional homozygous reciprocal translocating cations were isolated / the total to over 100. A number of intercrosses between homozygous translocations were made to identify translocated chromosomes but have not been cytologically analysed.

Seed-set data was also obtained on 22 of the reciprocal translocation in the heterozygous condition to determine their value for use in the genetics program (Table 9). The seed-set on the translocations in the heterozygous condition ranged from 31 to 80% of seed-set of the average on the 23DA and 23DB controls. This seed-set indicates that they will be useful in our genetics program.

A number of our multimarker gene lines were crossed on the tester set in 1978. The  $F_1$ s were planted in the greenhouse during the winter. The  $F_2$  will be planted in the field in 1979.

Table 9. Open-pollinated seed set on cytoplasmic-genic male sterile F<sub>1</sub> (23DA x homozygous translocation tester stocks) plants heterozygous for reciprocal translocation tester stocks.

1978 plot	t stock	Chromosome involved	Ave.
13119	51		.185
13120	51		.126
13121	56		.144
13122	56		.180
13123	56		.153
13124	52		.171
13125	52		.190
13126	52		.231
13127	53		.205
13128	53		.190
13129	3	AB	.110
13131	3	AB	.147
13132	3	AB	.144
13133	4	CD	.160
13134	6	CE	.202
13135	6	CE	.230
13136	7	CG	.288
13137	7	CG	.224
13138	7	CG	.295
13140	8	AC	.181
13141	8	AC	.130
13142	9	CG	.283
13143	11	DG	.189
13144	11	DG	.277
13145	15	AD	.233
13146	18	BE	.205
13147	18	BE	.171
13148	18	BE	.132
13149	18	BE	.170
13150	19		.199
13151	20		.120
13152	20		.181
13153	22		.198
13155	22		.140
13156	23		.252
13157	23		.169
13158	26		.185
13159	26		.228
13160	28		.251
13161	28		.189
13163	32		.146
13164	33		.249
13165	33		.126
13167	34		.223
13168	34		.169

Table 9 (con't). Open-pollinated seed set on cytoplasmic-genic male sterile F<sub>1</sub> (23DA x homozygous translocation tester stocks) plants heterozygous for reciprocal translocation tester stocks.

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1978 plot	t stock	Chromosome involved	Ave.
13169	34		.238
23DA-1	check		.261
23DA-2	check		.328
23DA-3	check		.396
23DB-1	check		.386
23DB-2	check		.376
23DB-3	check		.352
	5% LSD	.106	
	SE	.066	
	CV	31.51	

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Part K. Use of Radiation to Control the Reproductive Behavior in Plants.

Apomixis, seed production without fertilization of the egg would have tremendous potential in the production of hybrids.  $F_1$  hybrids are usually superior but they must be produced by repeated controlled crossing of parental lines in sexual species. An apomictic hybrid would fix heterosis since it perpetuates itself through identical offspring for successive generations. Apomixis would simplify hybrid seed production and increase the success of "wide" crosses.

Pearl Millet

Stubby head, a facultative apomict has been induced with a combination treatment of thermal neutrons and diethyl sulfate in pearl millet, a sexual species. Appropriate tests have shown that approximately 25% of the progeny produced by this mutant are of maternal origin. Ideally, one would want 100% of the progeny to be of maternal origin.

We are attempting to produce a plant that would be an obligate apomict by introducing a thermal neutron induced female sterile (fs) gene in the facultative apomict genotype. Theoretically, the plants homozygous for both genes would be obligate apomicts since the female sterile (fs) gene would destroy the sexual tissue leaving only the nucellar tissue to develop into aposporous embryo sacs to produce maternal embryos. The fs gene should also enhance apomictic development since cytological analyses indicate that it has aposporous embryo sac development. We have been unsuccessful in the past in recovering the double homozygote, apparently because of a close linkage.

In 1978 we searched for plants homozygous for both mutants among over 6700 plants from 288  $F_2$  and backcross progenies. We identified 94 plants as suspects and testcrossed them with a dominant red (R) or purple (P) mutant. These testcrosses were planted in flats in the greenhouse during the winter of 1978. The results in 1978 were more

encouraging since 18 testcrosses gave a high frequency of green plants indicating obligate apomixis. These green plants will be transplanted to the field to see if the phenotype identified in the field in 1978 is maintained.

Part L. Genetics of Radiation Induced Mutations.

The radiation breeding program at Tifton, Georgia has produced numerous mutations in pearl millet. Some such as trichomeless, early, facultative apomictic, and dwarfs have immediate economic possibilities. Whereas, others are valuable to the plant breeder and geneticist as genetic markers in the plant breeding program for developing and studying breeding procedures and genetic mechanisms, for making testcrosses and for identifying linkage (gene) blocks on specific chromosomes.

Each year we are evaluating the mutations for agronomic potential, gathering inheritance and linkage information, and conducting allelism tests to eliminate identical mutations that may have occurred as separate events.

The trichomeless (tr) mutant in pearl millet has been of special interest to us because of its drought tolerance and pest resistance. In 1978, we found (with the electron microscope expertise of Dr. Danny Akin) that the tr mutant has a more perfect cuticle which accounts for its lower transpiration of water (Crop Science 18:904-905, 1978, reprint attached).

In 1978, 22 mutagen induced chlorophyll deficient mutants in inbred 23B were intercrossed to test for allelism. All except for two were mutants controlled by different loci.

The large amount of genetic and linkage data collected in our program each year necessitated the need to develop computer programs that would

analyse and summarize the data in table form. Three programs were developed and are described in Crop Science 18:517, 1978 (reprint attached).

### Inheritance

A number of excellent new mutations have been induced in pearl millet. These characters will be very useful in mapping specific chromosomes and for use in genetic stocks. Inheritance data on 8 mature plant characters are summarized in Table 10. The inheritance data of two mature plant chlorophyll deficient mutants in pearl millet has been published in the Journal of Heredity 69:273-274, 1978. The linkage relationships of these two loci and with other loci are also reported. A reprint is attached.

### Linkage

The relationships and location of genes controlling various mutations are being established. The initial stages of the linkage program has involved intercrossing mutants in order to gain inheritance and repulsion linkage data. Recovery of double homozygotes from the F<sub>2</sub> populations will allow us to develop multiple gene tester stocks which will greatly speed up our linkage work. One such stock that we now have is dwarf-lazy-yellow node-smooth. The multiple gene stocks will also allow us to gather coupling and backcross linkage data which is much more efficient and accurate than repulsion data.

The 1978 linkage data (Table 11) has greatly added to our understanding of the relationships between a number of the radiation induced mutants. The data show the presence of 8 linkages as indicated by a significant linkage X<sup>2</sup> value. The independent relationships of 26 gene pairs were also observed.

Table 10. 1978 chi-square analyses on progenies from plants heterozygous for radiation induced pearl millet mutants.

Mutant	Total plants	Theoretical ratios	$\chi^2$ (P > .05)
Stubby head	2705	7 : 1	0.015
Bronze	573	13 : 3	0.075
Lazy	3796	13 : 3	0.018
Stripe	480	3 : 1	1.344
Necrotic leaf ends	164	3 : 1	.130
Mosaic	537	13 : 3	0.395
Branched head	294	3 : 1	1.020
Target spot	301	13 : 3	1.206

Table 11. 1978 chi-square analyses on linkage data from radiation induced pearl millet mutants.

Mutants	Number		df	Hetero.	Linkage	Phase <sup>+</sup>
	Families	Plants		$\chi^2$	$\chi^2$	
Branch head - bronze	4	294	9	1.13	23.24*	R
Purple - bronze	1	139			0.02	R
Dwarf - bronze	3	299	6	3.11	2.80	R
fs - bronze	1	139			0.65	R
tr - fs	1	94			0.12	R
yn - stubby head	1	94			1.07	R
stripe - stubby head	1	94			1.51	R
tr - target spot	1	90			0.20	R
stripe - fs	2	145	3	2.28	18.88*	R
fs - target spot	1	90			1.15	R
Red - tr	4	417	9	3.79	2.41	C
Red - lazy	8	815	21	4.68	12.21*	C
Dwarf - red	10	1051	27	9.36	29.36*	R
tr - lazy	6	551	15	3.03	0.25	C
Purple - tr	10	924	27	8.69	0.00	R
Purple - lazy	6	495	15	26.54	0.55	C
Purple - target spot	3	301	6	0.06	0.18	R
yn - target spot	3	304	6	3.24	3.43	R
lazy - fs	37	2784	108	82.75	1.03	R
Dwarf - fs	30	2887	87	25.29	1.49	R
Purple - dwarf	30	3037	87	129.62	50.51*	C
Purple - fs	19	1866	54	28.99	0.53	R
tr - fs	33	2816	96	38.87	0.08	C
Dwarf - lazy	39	3807	114	458.51	257.09*	C
stubby head - fs	45	2705	132	65.55	257.83*	R
tr - lazy	25	2452	72	17.05	0.99	C
necrotic leaf ends - fs	3	164	6	1.58	0.17	R

Table 11(con't). 1978 chi-square analyses on linkage data from radiation induced pearl millet mutants.

Mutant	Number		Hetero.		Linkage	
	Families	Plants	df	$\chi^2$	$\chi^2$	Phase <sup>+</sup>
Mosaic - yn	5	537	12	5.21	63.12*	R
Mosaic - tr	5	537	12	2.20	0.57	R
Mosaic - lazy	5	537	12	5.39	1.50	R
Lazy - yn	10	928	27	4.34	4.94	C
Dwarf - yn	17	1680	48	27.43	4.61	C
Purple - yn	18	1803	51	24.99	0.26	C
yn - stripe	5	480	12	19.75	80.67*	R
Dwarf - tr	25	2527	75	16.63	0.03	R

<sup>+</sup> C = coupling, R = repulsion

\*  $P < .05$  for independence

Part M. Response of Pearl Millet Pollen to Gamma Radiation.

A number of mutants in inbred Tift 23B have been recovered from mutagen treated material in the irradiation breeding program at Tifton. These mutants are essentially isogenic (genetically identical except for the mutated loci). They are excellent materials to use in various genetic studies.

A better understanding of the genic and genotypic response of plants to radiation would be of value to plant breeders and geneticists. It would allow them to use radiation more effectively in accomplishing breeding and improvement objectives.

Preliminary data in 1975 indicated a differential response of pollen from isogenic inbred 23 pearl millet mutants to gamma irradiation. Data was not collected in 1978 because the mutants flowered at different times due to droughty conditions. In 1979 the mutants will be grown in the greenhouse.

Part N. Nature of Morphological Changes in Sterile Triploid Bermudagrass on Golf Courses.

The "Tif" series of bermudagrasses are sterile and triploid (27 chromosomes) and is grown on most of the golf courses throughout the Southern and Southeastern United States. Mr. James B. Moncrief, of the U.S. Golf Association Green Section has observed many offtype plants on certain golf courses. He has been collecting these plants and sending them to us for cytological analysis and further evaluation.

Twenty-seven and 7 offtype plants of 'Tifgreen', 'Tifway', and 'Tifdwarf' were established in 8' x 8' plots in 1977 and 1978, respectively. Visual observations indicated that most were different from their parental clone (hybrid) which establishes that the changes

are probably genetic or chromosomal. Chromosome counts for each clone was obtained from roottips. Most of the clones had 27 chromosomes which shows that the changes in plant phenotype are genetic. A survey was begun in 1978 by Drs. Monty Moncrief and Bud White to see if there is a relationship between number of mutants observed and the pesticides used on golf courses. This survey has not been completed.

Although most of the mutants are less desirable than the parental clone, a few do have improved turf characteristics. The mutants are being subjected to close clipping conditions for further evaluation.