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

ANNUAL PROGRESS REPORT
FOR THE PERIOD
NOVEMBER 1, 1979, TO OCTOBER 31, 1980

MASTER

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PREPARED FOR THE
U. S. DEPARTMENT OF ENERGY

UNDER CONTRACT NO. DE-AS09-76EV00637

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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_1 sterile cytoplasm used in producing hybrid millet throughout the world. Tift 239 and Tift 13 are maintainers for A_2 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_1 hybrid when crossed on Tift 23A₁.

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 presented 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.

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 data showed 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 could not 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.

In 1979, 20 of the most promising selections (based on 1978 analyses) from irradiated material were again replicated for further selection. Also eleven introductions with higher protein and lysine were grown. These were sent to the International Atomic Energy Agency in Vienna, Austria but results of analyses have not been received at the writing of this report.

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

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 outcrossing 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 assessment of grain size and composition.

The results of this study were published 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 reprint appears with the 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 by 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 above-ground 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.

Data collected in the past year warranted the preparation of Table 1 summarizing the overall performance of the nine superior mutants and their parent clones. The excellent performance and extra frost tolerance of Tifway mutant 72-126 has caused Southern Turf Nurseries to use it in replanting the University of Georgia football field in 1980. It will probably be released as Tifway 2 in 1981.

In the spring of 1979, we sent plugs of the grasses listed in Table 1 and 22 of our better hybrids and selections to Dr. Ralph Engle,

THE COMPARATIVE PERFORMANCE OF TIFGREEN, TIFDWARF, AND TIFWAY BERMUDAGRASS AND NINE OF THEIR MUTANTS INDUCED BY EXPOSURE TO GAMMA RAYS FROM COBALT 60

	Maximum Height-inches	Height-inches after 4 wks	Seed head abundance	Sod density rating	Percent cover 4/28/75	Weed rating 2" cut	Turf quality May	Turf quality July	Turf quality October	Sod webworm damage %	Nematodes 150 cc of soil after 3 years				Frost Response Athens, Ga. 1978 Ratings made			Growth & cover 4/10/80
											Root knot	Ring	Stubby	Sting	11/22	12/11	12/13	
1. Tifgreen	8	4	4	1	97	3.7	2.0	1.1	1.0	0	465	127	29	69	2.5	1.5	1.7	6.5
2. Tifgreen mutant 72-16	12	9	4	1+	98	1.0	1.5	1.0	1.0	0	0	90	20	290	5.5	5.0	1.7	1.0
3. Tifgreen mutant 72-49	6	2	1	2	70	4.0	1.7	1.0	1.0	8	1260	260	80	0	3.0	1.5	1.0	9.0
4. Tifgreen mutant 72-54	12	7	10	2	100	1.7	1.0	1.0	1.0	0	2360	480	60	0	4.2	3.5	1.0	2.5
5. Tifgreen mutant 72-59	15	8	8	1+	100	1.3	1.0	1.0	1.0	0	0	190	0	0	4.0	3.5	2.8	2.5
6. Tifdwarf	2	2	2	4		5.0	2.0	2.8	2.2	63	411	153	27	16	3.5	2.5	1.5	7.0
7. Tifdwarf mutant 70-45	3	2	2	1		5.0	1.5	1.0	1.0	47	380	860	20	80	2.5	2.2	1.0	6.5
8. Tifdwarf mutant 70-47	4	3	2	1		5.0	1.2	1.0	1.0	40	140	140	0	0	1.2	2.5	1.2	5.0
9. Tifway	13	8	4	1	100	1.3	1.0	1.2	1.2	0	34	223	18	7	4.0	4.2	1.7	3.0
10. Tifway mutant 72-114	7	3	2	2	85	3.0	3.0	1.0	1.0	0	0	160	0	20	3.7	4.0	2.5	3.5
11. Tifway mutant 72-117	14	9	4	1	100	1.7	1.0	1.0	1.0	0	0	10	0	0	5.7	5.0	2.5	1.5
12. Tifway mutant 72-126	16	8	4	1+	100	1.0	1.0	1.0	1.0	0	0	160	40	0	5.0	5.5	4.0	1.5

Management has been minimal to indicate those grasses able to provide good turf with reduced maintenance. Thatch has been removed each spring and 2,4-D (1 lb/A) has been applied to remove winter weeds. Fertilizer (a 4-1-2 N-P₂O₅-K₂O) has usually been applied to give 100 lbs. of N/A in mid March and one or two applications later in the season. Last year Bromacil (Hyvar) was sprayed between plots to keep them separated. It moved into the edge of some plots with heavy rains and accounted for dead spots along the side of some alleys. Otherwise, it did an excellent job of keeping the alleys bare and the plots separated.

Basagram proved to be an effective selective herbicide to burn back nutsedge in bermudagrass turf. It did not eliminate the nutsedge. Generally herbicides, fungicides, insecticides, and nematicides have not been applied. If applied, all plots received the same treatment.

In 1979, the west third of each plot was cut at a height of 2", the middle third was cut at 1/4" and the east third was cut at 3/4". Roundup has been used to kill out mixture resulting from an inadequate kill with methyl bromide.

Selected measurements and ratings are presented above to describe the performance of each grass.

RATINGS - Grasses rating 1 have the fewest heads, the best sod density, turf quality, growth and cover and the fewest weeds. Frost response ratings made by Dr. Tim Bowyer give grasses with the least frost tolerance a rating of 1 and those with the greatest tolerance a rating of 5.0. Ratings made 12/13 following 4 days of freezing lows.

Rutgers University, New Jersey and to the Pine Valley Country Club in New Jersey where they will be evaluated for winter hardiness under golf course management. These grasses were also planted on several North Carolina golf courses by Bud White, USGA Green Section Agronomist. Again the main objective is to detect winter hardiness differences under golf course management.

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 winter hardiness 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°C . All plants from non-irradiated stems winterkilled. The four surviving plants are

still being treated in replicated plantings for winter hardiness. 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 that 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, winter hardy. 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°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 $5 \frac{3}{4}$ 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.

On the late afternoon of June 6, we cut and baled 18 bales of green sprigs from Coastcross 1-M3 mutant and 10 bales from Coastcross-1. These were taken to Oak Ridge, Tennessee in a 2-ton truck from 5:00 a.m. to 2:30 p.m. on June 7. They were subjected to 7,000 r from 3:00 p.m. to 10:00 p.m., were loaded on the truck at 5:00 a.m. and delivered to the Georgia Mountain Experiment Station at 9:30 a.m. on June 8. They were then planted and were sprayed with 2,4-D at 2 lb/A to control weeds. The planting became well established during the summer and went into the winter in good condition. An unusually mild winter with -3°F with snow cover allowed too much of the grass to survive to get a satisfactory screening for possible winter hardy mutants.

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°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" has been accepted by the Journal.

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 has 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%. The yield data presented in the 1978 report showed 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 hybrid between Tift 23DA and Tift 239DB mutants were included in the 9 x 9 yield trial. 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 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.

During 1979, we prepared a paper entitled "Hybrid Vigor in Forage Yields of Crosses between Pearl Millet Inbreds and their Mutants". This paper has been accepted by the editors for publication in Crop Science. A copy of the manuscript is included with the reprints of this report.

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.

On July 9, 1979, we (in cooperation with Dr. Phil Utley, animal scientist; Dr. Paul Martin, entomologist; and Dr. Warren Monson, agronomist) established two 0.81 ha pastures of each of the isogenic bloom and bloomless sudangrass lines. From 8/15/79 to 10/10/79, these pastures were grazed with both steers and heifers. Due to the late planting and non-availability of irrigation, poor stands were obtained. Consequently, unreliable grazing data was obtained.

Fewer fall armyworm were observed on the bloomless sudan compared to the bloom line. A similar observation was made for the greenbug by Peiretti and Weibel in 1976.

The bloomless characteristic deserves further evaluation especially in a sorghum-sudangrass hybrid. A bloomless cytoplasmic-genic male sterile sorghum has been developed and will be used to produce a bloomless sorghum-sudangrass hybrid for further testing.

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 23A 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 to obtain 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 three 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 2). The frequencies of mutants observed in this M₁ material in 1977 are recorded in Table 2.

On July 20, 1978, M₂ progenies of more than 50 plants were grown from open pollinated seed of each M₁ mutant observed in 1977. Since these M₁ mutants occurred in a field of Tift 23DB with all plants except the mutants shedding pollen, open pollinated seed would be in

Table 2. 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 ₁ heads produced	32,800	13,000	22,300	18,000
M ₁ partially male sterile head mutants	13	24	9	22
M ₁ whole head male sterile mutants	20	36	21	9
<u>Seed planted 7/20/78</u>				
M ₂ progenies with some partially male sterile plants	8	21	10	11
M ₂ progenies with all plants cytoplasmic male sterile	2	5	4	4
M ₂ completely cytoplasmic male sterile progenies per 16,000 M ₁ heads	1	6.2	2.9	3.6

effect Tift 23DA mutant maintained with Tift 23DB pollen. Table 2 shows that only one-third to one-fourth of the M_1 mutants produced progenies with some partially male sterile plants.

Of particular significance is the number of progenies in all treatments that were as completely cytoplasmic male sterile as 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 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 2 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. Many of these hybrids were grown out in the late summer of 1979 and were rated for fertility restoration. Usually 12 to 15 plants of each hybrid were grown and assessed. Those rated part fertile ranged from plants with a few anthers shedding pollen to plants within a row that ranged from sterile to fertile. A summary of these ratings appears in Table 3.

The "2-open" hybrid progenies were from open pollinated heads in the field when most plants were the pollen shedding inbred 2 (23DB), the inbred in which the mutants had been induced. All other hybrids were made by hand pollinations.

The number of progeny examined ranged from 13 for the check to 52 for hybrids involving mutants from the 200 ppm streptomycin treatment.

Table 3. The fertility of hybrids between cytoplasmic male sterile mutants induced by streptomycin or mitomycin and eight pearl millet inbreds.

Mutagen	Hybrid fertility	Numbers of sterile mutant hybrid progenies that were sterile, fertile or partially fertile when a sterile mutant was pollinated with inbred no.								
		2-open	2	4	5	7	8	9	10	28
Check (23DA)	Fertile				11	1	3	1		
	Part f				2				1	5
	Sterile	13	3	2						
Strepto- mycin 200 ppm	Fertile		1		25	3	5	4	1	1
	Part f				5	5	6			17
	Sterile	52	14	13	1	2			3	
Strep 1 500 ppm	Fertile				26	3	9	3	2	3
	Part f				2	2	5	1	1	10
	Sterile	43	19	12	3				1	
Mito- mycin 500 ppm	Fertile				18		16	4	1	1
	Part f	2	1		13		1		4	12
	Sterile	27	11	10	9		1		1	1

Hand pollinations with inbred 2 gave results similar to those obtained from "2-open" as might be expected. Likewise, inbred 4 (23DBS) proved to be a good sterility maintainer for all mutants.

Inbred 5, a very good fertility restorer for 23DA when hybrids flower during the summer still restored fertility quite well in the check in the fall even though fertility restoration was poorer at that time. It is interesting that it restored fertility less effectively for the streptomycin mutants and much less effectively for the mitomycin mutants. For mitomycin mutant No. 1962, only one of nine hybrids with inbred 5 was fertile, 3 were partially fertile and 5 were sterile. These results suggest that induced mutant 1962 is different than 23DA.

Although the numbers are limited, inbreds 7, 8, and 9 restored fertility well for 23DA. Only 9 performed as an excellent fertility restorer for the induced mutants. Inbreds 10 and 28 performed largely as partial fertility restorers for both the 23DA check and all male sterile mutants.

The fertility of the hybrids just described, suggests that the induced mutants from 23DB are similar to 23DA but some appear to be significantly different.

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°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 4 were applied to 3-row plots 5.6 m long in triplicate on May 23 and June 23, 1978. The grass grew well throughout the summer with no insect or disease attacks.

One 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 4 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 in 1978 (Table 4). 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.

In 1979, this test was fertilized as in 1978 with all of the fertilizer for treatment 1 and half of it for treatments 2, 3, and 4 applied in late March and the other half in June. Because there was so much growth by late July, we decided to cut the test twice instead

Table 4. 1979 biomass production of Merkeron napiergrass set as rooted single stem cuttings on .9 m centers 5/7/78.

Treatment	Fertilizer applied			Dry matter yield		Total
	Kg/ha of			T/ha		
	N	P ₂ O ₅	K ₂ O	7/31/79	12/11/79	
1	120	60	120	24.7	13.1	37.8
2	360	180	360	20.8	9.2	30.0
3	480	240	480	17.2	9.3	26.5
4	480	240	480	28.5	16.5	45.0
5% LSD				9.0	NS	10.9

Fertilizer was applied to 6 x 3 plant plots. Yields were taken on each plant and the above yields are based on the performance of the 4 plants in the center of each plot.

The total dry matter harvested from the whole test eliminating a row of plants all around the outside of the experiment was 26.4 T/ha.

of once as we did in 1978. This gave us yields up to 28 T/ha of dry matter (Table 4). The second cutting taken December 11 yielded much less than expected, giving total yields in excess of 30 MT/ha for most of the treatments. The yield of treatment 3 was low because several plants receiving that treatment were nearly killed with the harvesting equipment in 1978. We have found it necessary to modify our harvesting equipment so that the weighing trailer that follows the chopper will not damage harvested plants that it passes over.

Treatment 1, receiving 120-60-120 of $N-P_{2O_5}-K_2O$, gave a total yield of 37.8 MT/ha in 1979 compared with 38.7 MT/ha in 1978. This treatment seems to be enough to nearly maximize yields and produce biomass with 1% N and K and 12% P. Had we cut this treatment only one time, I believe it would have yielded much more biomass than that recorded.

There is a need to ascertain the effect of cutting frequency on the yield of this grass. We plan to divide this test into 3 units in 1980, cutting one-third 3 times, one-third 2 times, and one-third only once in an effort to throw some light on this question.

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 data showed that all mutants except bmr 17 already had higher forage quality one month after planting. There were generally non-significant differences in percent dry matter between isogenic normal and bmr lines. The data also showed that the bmr lines (except for bmr 12) were higher in total sugars than the normal lines. The data suggested that total sugars had no effect on percent IVDMD. This is supported by our earlier work with total sugars in pearl millet.

Additional analyses on the 1978 samples are reported for lignin (Table 5), percent starch, and percent nitrogen (Table 6). The lignin content was lower for both bmr lines at all harvest dates which accounts for the higher IVDMD of the bmr lines. Even though the differences were not significant at the first two harvest dates due to few degrees of freedom, we believe that they are real differences. The normal lines generally has higher starch content than the bmr lines (except for line 18) but these differences were significant only for line 12. We also selected lines with high (line 14) and low (number 17) starch and analyzed them for percent total nitrogen.

Table 5. % Lignin in brown-midrib and normal forage harvested in 1978.

Entry	% Lignin in forage harvested on:			
	6/19	7/21	8/07	$\bar{x}$
bmr 12	2.15	2.75	3.00	2.63
Bmr 12	3.20	3.85	4.00	3.68
bmr 18	2.05	2.70	2.75	2.50
Bmr 18	2.75	4.00	4.40	3.72
Entry	NS	NS	*	*
SE	0.20	0.42	0.15	0.13
CV	11.0	12.6	6.0	6.0
5% LSD			0.67	0.60

Table 6. 1978 analyses on brown-midrib and normal sorghum for % total nitrogen and % starch.

	% Starch	% Total Nitrogen (1978)				
	8/7/78	6/19	7/05	7/21	8/07	$\bar{x}$
bmr 6	3.5					
Bmr 6	3.9					
bmr 18	2.1					
Bmr 18	1.9					
bmr 14	5.3	3.34	2.14	1.39	1.07	1.98
Bmr 14	5.6	3.29	2.15	1.57	0.98	1.87
bmr 17	1.8	3.07	1.93	1.46	1.02	2.00
Bmr 17	2.7	2.60	1.74	1.22	0.84	1.60
bmr 12	2.2					
Bmr 12	4.6					

ANOVA						
Rep.	NS	NS	NS	NS	NS	NS
Mutant	NS	**	NS	NS	NS	*
Inbred	**	NS	NS	NS	NS	NS
M x I	NS	NS	NS	NS	NS	NS
S	1.33	0.21	0.29	0.19	0.15	0.12
CV	39.0	7.0	14.8	13.4	14.9	6.47
5% LSD						
Inbreds	1.0	0.30				0.17
Mutant						

There were no large differences between lines, however, the bmr mutants tended to have higher nitrogen.

In 1979, we repeated the 1978 experiment. However, in addition to the quality test, we also estimated yield. Dry matter yields and percent dry matter are reported in Table 7. The normal lines out-yielded the bmr lines (except for line 6) but these differences were significant only for lines 12 and 14. The bmr lines yielded less because they appear to be more susceptible to insects (to be discussed later). There were no consistent differences in percent dry matter.

The large differences detected in 1978 for percent IVDMD at one month after planting were not detected until 7 weeks after planting in 1979 (Table 8).

The higher percent IVDMD for the bmr lines was then maintained for the remainder of the season. These data still show that the higher quality of the bmr lines is expressed at a relatively early date, making this characteristic important in a grazed forage sorghum. In 1979, there were again no consistent differences in favor of either bmr or normal lines as in 1978, however, normal 12 was higher in total sugars than bmr 12 at all harvest dates except on 7/13 (Table 9).

In 1979 (in cooperation with Dr. Joe Johnson, dairy scientist; Dr. Paul Martin, entomologist; and Dr. Warren Monson, agronomist) we also planted 1 ha of each bmr 12 and normal 12 for the production of silage. The sorghum planted on June 6 was fertilized with 570 kg of 5-10-15 and later sidedressed with 114 kg of ammonium nitrate. It was harvested in August and ensiled. This silage was fed to dairy animals, however, the data has not been analyzed at the writing of this report.

Table 7. Dry matter yield and % dry matter of 5 isogenic bmr sorghum lines grown in 1979 (I75).

Line	g/plot	Dry matter			% Dry matter on:	
		Signif.	%	Signif.	7/13	Signif.
bmr 6	1153	NS	28.8	NS	10.2	NS
normal 6	1021		24.9		9.9	
bmr 18	1573	NS	30.9	NS	9.2	NS
normal 18	1928		29.9		9.2	
bmr 14	720	*	27.2	**	10.4	NS
normal 14	2731		33.5		10.4	
bmr 17	1728	NS	31.8	NS	9.6	NS
normal 17	2001		31.0		10.0	
bmr 12	1336	*	30.2	NS	9.2	*
normal 12	2384		31.4		11.2	

ANOVA

Entry	**	NS	NS
SD	496	2.83	0.53
CV (%)	29.9	9.5	5.3
5% LSD	715		

Table 8. IVDMD of 5 isogenic bmr lines harvested at four dates in 1979 (I75 field).

Line	IVDMD on Harvest Date							
	7/13	Signif.	8/07	Signif.	8/28	Signif.	10/3	Signif.
bmr 6 ⁺	58.8		70.0		68.0		57.8	
Normal 6	59.6	NS	69.1	NS	63.3	*	54.5	NS
bmr 18	60.5		67.3		66.1		57.8	
Normal 18	56.2	*	64.3	*	64.9	NS	55.1	NS
bmr 14	59.0		71.1		70.3		63.5	
Normal 14	59.7	NS	66.1	*	64.8	*	59.3	*
bmr 17	58.9		67.3		66.0		60.4	
Normal 1	59.9	NS	64.0	*	62.9	NS	56.3	*
bmr 12	59.5		68.9		67.4		65.5	
Normal 12	61.1	NS	64.3	*	62.7	*	58.8	*

ANOVA				
Entry	NS	**	**	**
SD	2.6	1.8	2.6	2.4
CV (%)	4.4	2.7	4.1	4.1
5% LSD		2.7	3.9	3.5

Table 9. % total sugars in brown-midrib forage harvested in 1979.

Entry	% total sugar in forage harvested on:				
	7/13	8/7	8/28	10/3	$\bar{x}$
bmr 6	2.6	19.3	22.3	13.3	14.4
Normal 6	2.8	15.6	26.7	12.9	14.5
bmr 18	2.4	18.0	24.7	14.2	14.8
Normal 18	2.7	20.3	22.8	16.9	15.6
bmr 14	3.0	18.3	36.7	15.8	18.4
Normal 14	2.7	21.6	28.1	21.0	18.4
bmr 17	2.5	17.6	27.3	15.2	15.7
Normal 17	2.6	21.9	30.0	13.5	17.0
bmr 12	2.9	24.1	28.3	17.3	18.2
Normal 12	2.6	32.9	44.4	22.5	25.6
Entry	NS	NS	**	NS	*
SE	0.13	4.6	1.9	2.6	1.5
CV	7.0	31.3	9.4	23.4	12.2
5% LSD	0.4	14.8	6.2	23.4	4.8

Table 10. Intensity or damage in Brown Midrib-12 and Normal-12 forage sorghum (insecticide treated and untreated) of or from thrips, corn leaf aphids, lesser cornstalk borer, fall armyworm, sorghum midge, headworms and insecticide.

Cultivar	Insecticide Treated ^{1/}	Untreated (Range)	$\bar{x}$ (treat and untreated)
<u>Thrips/5 plants</u>			
BMR-12	15.30	25.30 (7-44) ^{2/}	20.30
Normal-12	14.20	16.80 (5-29) ^{2/}	15.50
<u>Corn leaf aphids/5 plants</u>			
BMR-12	0.20	9.80 (0-55) ^{2/}	5.00
Normal-12	0.00	3.25 (0-13) ^{2/}	1.75
<u>Lesser cornstalk borer damage/10 plants</u>			
BMR-12	0.20	1.65 (1.30-2.00) ^{3/}	0.92
Normal-12	0.22	0.82 (0.80-0.85) ^{3/}	0.52
<u>Fall armyworm/10 plants</u>			
BMR-12	1.07	1.52 (1.00-3.05) ^{3/}	1.30
Normal-12	1.03	1.50 (0.15-2.85) ^{3/}	1.26
<u>Fall armyworm damage rating^{4/}</u>			
BMR-12	4.73	5.65 (5.25-6.40) ^{3/}	5.19
Normal-12	3.01	4.13 (3.35-5.10) ^{3/}	3.57
<u>Sorghum midge + sorghum webworm damage rating^{5/}</u>			
BMR-12	5.29	4.40 (2-7) ^{2/}	4.84
Normal-12	1.95	1.85 (1-6) ^{2/}	1.90
<u>Headworms/10 heads</u>			
BMR-12	14.30	--	(14.30)
Normal-12	14.20	--	(14.20)

(Cont'd.)

Table 10. (Cont'd.)

Cultivar	Insecticide Treated ^{1/}		Untreated (Large)		$\bar{x}$ (treat and untreated)
		<u>Phytotoxicity rating^{6/}</u>			
BHR-12	3.32	-	2.65 (-)	=	(0.67) ^{7/}
Normal-12	2.60	-	2.20 (-)	=	(0.40) ^{7/}

1/ Sixteen applications from planting to stage 7.

2/ Twenty "readings" on 1 date.

3/ From 2-3 sample dates.

4/ 0 = no detectable damage, 1 = some "pinhole damage", 5 = several large holes or several leaves, and 9 = damaged to the extent that the plant was not "recoverable".

5/ Twenty 10-row-ft ratings from each treatment --1=no damage; 5 = 31-40% damage; 9=91-100% damage (Frederikson et al. 1977).

6/ Twenty 10-row-ft ratings from each treatment --0=no phytotoxicity, 5=severe purpling, leaf burn; 10=plant completely dead (Clement and Sidwell 1978).

7/ Difference is treated and untreated.

Data on insect damage (Table 10) tends to indicate that the bmr lines are more susceptible to insects especially at early growth stages. Since even the normal line is quite susceptible, we believe that if the bmr genes are transferred to locally adapted lines, that the damage due to the bmr genes can be reduced to a non-significant level.

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). Gildenhuys and Brix (Agroplantae 1:77-84, 1969)

found that they could not transfer desired traits of napiergrass to pearl millet with three backcrosses 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.

In 1979, we established 1045 backcross progeny from the irradiated crosses. Almost all of the progeny appeared to be normal pearl millet ($2n = 14$) and much to our surprise, all were cytoplasmic male sterile indicating that the original inbred 23 maintainer genome was recovered. We did see some plants with various degrees of rust resistance indicating that some of the napiergrass genome was transferred. However, these were not maintained because of the male sterility. We hope to solve the male sterility problem in 1980 by backcrossing the irradiated 6x pollen to a fertile line with a recessive marker so that backcross hybrids can be distinguished from selfs. A number of Tift 23DB tr x irradiated 6x pollen crosses were made in the fall of 1979. These will be planted in the field in 1980.

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 identification 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 translocations were isolated bringing the total to over 100. Seed-set data was also obtained on 22 of the reciprocal translocations in the heterozygous condition to determine their value for use in the genetics program. The seed-set on the translocations in the heterozygous condition ranged from 31 to 80% of seed-set on the average on

the 23DA and 23DB controls. This seed-set indicates that they will be useful in our genetics program. A number of intercrosses between homozygous translocations were made to identify translocated chromosomes. In 1979, 25 intercrosses were cytologically analyzed. These confirmed some of our past established relationships and also gave us additional information on new translocated chromosome combinations.

New information on the linkage of gene loci with specific translocation stocks was obtained in 1979 as reported in Table 11. The data indicates that tr and lazy genes are located on the B chromosome. The d_1 is located on the C chromosome and red is located on either the A or C chromosomes.

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

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.

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

Table 11. 1979 chi-square analysis on data for linkage relationships between translocation stocks and radiation induced mutants.

t-stock - mutant	Number		Heterogeneity		Linkage
	Families	Plants	df	χ^2	χ^2
t4 - Purple	2	356	3	1.22	4.08
t3 - Purple	1	140			4.67
t18 - tr	2	261	3	.11	16.31*
t8 - tr	3	373	6	.97	1.65
t4 - tr	1	163			.02
t3 - tr	3	445	6	4.72	16.63*
t18 - lazy	2	261	3	.25	52.21*
t8 - Red	2	235	3	.10	9.77*
t3 - Red	2	305	3	.80	.14
t8 - d ₁	2	103	3	3.93	2.62
t3 - d ₁	2	127	3	1.76	15.48*
t18 - Purple	2	235		.83	1.74
t18 - yn ₁	4	496	9	3.02	.97
t8 - yn ₁	1	138			.97
t4 - yn ₁	3	519	6	1.96	7.08
t3 - yn ₁	2	280	3	.23	.58

* $P < .05$ for independence

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 aposporus embryo sacs to produce maternal embryos. The fs gene should also enhance apomictic development since cytological analyses indicate that it has aposporus 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 F₂ 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 were transplanted to the field in 1979 to see if the phenotype identified in the field in 1978 is maintained. All of the plants had a normal head type (different from the phenotype identified in 1978) indicating that they did not reproduce by apomixis. There is apparently a close linkage between the fs and stubby head genes which prevents recovering the double homozygote. Possibly a more fruitful approach with the facultative apomict will be to further irradiate it in hopes of producing an obligate apomict.

In 1979, we were successful in crossing pearl millet (sexual) with both fountaingrass (apomictic), Pennisetum setaceum and P. orientale (apomictic). The interspecific hybrids are also apomictic and male

sterile. Attempts are now being made to double the chromosome number of the interspecific hybrids to produce the amphidiploids in hopes that they will be male fertile so that the pollen can be irradiated and backcrossed to pearl millet. These hybrids should be an important bridge for transferring segments of the chromosomes with apomictic genes from the wild species to pearl millet.

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.

An outstanding early mutation induced in inbred 23B (flowers 45 days after planting which is 40-50 days less than normal inbred 23B) has potential for grain production and as an early cytoplasmic male female parent for producing early grain type hybrids. Inbred 23 has excellent combining ability. To test if inbred 23 early still has good combining ability each 23A early, 23A and 23DA were crossed with some of the best male parents. These hybrids will be tested in 1980.

Many other early mutations have also been recovered from our mutation breeding program partially supported by this contract.

One-hundred-thirty-seven of these were crossed with the early 23A (mutant line) to test for allelisms and to test for hybrid combinations with good grain potential.

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_2 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 1979 linkage data (Table 12) has greatly added to our understanding of the relationships between a number of the radiation induced mutants. The data show the presence of 4 linkages as indicated by a significant linkage X^2 value. The independent relationships of 12 gene pairs were also observed.

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.

Table 12. 1979 chi-square analyses on linkage data from radiation induced pearl millet mutants.

Mutants	Families	Plants	Heterogeneity		Linkage	
			df	χ^2		Phase [†]
Red - fs	1	135			.51	C
Red - laxy	1	135			8.40*	C
Red - lgn ₁	2	245	3	5.34	.43	C
Purple - lazy	1	154			5.06	C
tr - lgn ₁	2	245	3	.06	.22	C
lazy - yn ₁	3	415	6	3.80	2.34	C
lazy - fs	8	940	21	14.13	3.18	C
lazy - tr	2	261		.02	1.84	C
Red - tr	6	786	15	11.21	.03	C
d ₁ - fs	7	803	18	3.50	.78	C
d ₁ - tr	6	785	15	2.14	9.49*	R
d ₁ - lazy	8	959	21	161.79	3.39	R
d ₁ - yn ₁	2	202	3	.41	4.07	C
d ₁ - lgn ₁	2	245	3	1.01	10.07*	R
d ₁ - Red	6	785	15	7.43	110.86*	R
d ₁ - Purple	2	202	3	.46	5.72	C

† C or R - Coupling or repulsion, respectively

* P < .05 for independence

Preliminary data in 1975 indicated a differential response of pollen from isogenic inbred 23 pearl millet mutants to gamma irradiation.

In 1979, four isogenic lines (trichomeless, tr; red, R; purple, P; white leaf tip, wlt; yellow, yn; and dwarf, d) were crossed with Tift 23B in the field. These F_1 s were planted in the greenhouse along with the mutant lines. Pollen from the F_1 s was irradiated and backcrossed to the mutant lines and also used to pollinate the respective F_1 s (essentially a self with the pollen irradiated). Paired non-irradiated crosses were made at the same time. The irradiated backcross progeny will be planted in 1980 to detect differential sensitivity of the marker genes to radiation.

Part N. Nature of Morphological Changes in Sterile Triploid Bermuda-grass 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.

In 1977, 1978, and 1979, 56 offtype plants were established in 2.5 m x 2.5 m plots. Visual observations indicated that most of these offtypes were different than their parental clone (hybrid). Fifty had 27 chromosomes indicating that these offtypes originated by mutation. Two had 18 chromosomes and 4 had 36 chromosomes, both of which probably resulted from mixtures with common types. The new mutated offtypes are being evaluated for turf potential under clipping and for nematode resistance in cooperation with Dr. A. W. Johnson, Research Nematologist.