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EVALUATION OF TRIPLOID AND HEXAPLOID DERIVATIVES FROM *Pennisetum glaucum* x *P. purpureum* CROSSES AT THE BEEF RESEARCH UNIT, UNIVERSITY OF FLORIDA

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ABSTRACT

Cv. 'Mott' dwarf napiergrass has unusually high forage quality for a tropical grass (Sollenberger et al., 1988). Mott has been hybridized with pearl millet, and four of the best triploid F_1 progeny were selected for comparison with Mott and other non-dwarf Pennisetums. This five year study compared the dry matter yield of seven lines of *Pennisetum*, and also measured *in vitro* organic matter digestibility (IVOMD), crude protein (CP) and neutral detergent fiber (NDF).

The chromosome number of a triploid F_1 hybrid was doubled and three of the hexaploid progeny were evaluated at the Beef Research Unit in 1988 and 1989. In general, the hybrids showed similar IVOMD to Mott, but had increased leafiness and lower fiber than the tall napiergrass varieties.

INTRODUCTION

The theme of the meeting, "Germplasm and Biotechnology in Crop Improvement", corresponds with some of our recent work on hybrids between pearl miller, *Pennisetum glaucum* and napiergrass, *P. purpureum*. Napiergrass is a very diverse genus, and is one of the highest-yielding tropical and subtropical herbaceous biomass species. It has been targeted for genetic improvement by both plant-breeding and molecular methods. In this paper we report two experiments conducted in the field at the Beef Research Unit, University of Florida and adjunct experiments with restriction fragment-length polymorphisms (RFLP's) conducted in the Plant Science Laboratory, University of Florida. RFLPs are DNA fragments that can be detected by specific probes. They are useful in characterizing *Pennisetum* because they are observed directly and do not depend upon transcription or translation, nor are they influenced by environmental conditions. They are inherited as simple co-dominant Mendelian alleles, have heritabilities of 100%, and can be mapped into linkage groups and assigned to chromosome locations as with other genes (Smith et al., 1989).

MATERIALS AND METHODS

Experiment 1:

Germplasm for the five-year trial conducted 1985-89 included seven different lines. Three of the lines were tetraploid napiergrasses, viz. PI 300086, cv. Kinggrass; PI 517947, cv. Mott; and N-128, a vigorous, but unreleased tetraploid line. The other four lines, S3, S4, S37, and S38 in the test were triploid F_1 hybrids which originated from crosses of Mott to Tift 23DA pearl millet. Three of the sister hybrids were similar in height, leaf form and shape, and other characteristics, but the S3 hybrid was much shorter than the other lines (Rajasekaran et al., 1986). All planting stocks were generated by vegetative propagation. Dry matter forage yields, persistence, IVOMD, CP, and NDF were measured. Four replications in a randomized block design were harvested in July and December, 1985, 1986 and 1989, with single harvests made in December 1987 and 1988.

Experiment 2:

An S3 plant with its chromosome number doubled from triploid to hexaploid provided by Wayne Hanna, and superior hexaploid progeny were selected and added to the germplasm being tested. In a new experiment conducted 1988-89, these three new hexaploid selections, designated 360, 362 and 506 were compared with the three triploids, S3, S4, S41 and also with the tetraploid parent, Mott. Six replications in a randomized block design were harvested in December 1988, and again in July and November 1990. Data as described for experiment 1 was collected. Development and Use of RFLP Markers: Plant material of Mott, PI300086, pearl millet inbred lines Tift 23DA and Tift 239DB, and interspecific and intraspecific progenies between the parental lines were subjected to RFLP analysis. Genomic DNA was isolated according to procedures already reported (Smith et al., 1989). The genomic DNA was digested with EcoRI and HindIII restriction endonucleases and Southern blots were prepared as described by Maniatis et al. (1982). DNA hybridization and autoradiography was conducted as previously reported by Smith et al. (1989). Probes were made from clones of a random genomic clone library by ligating DNA of PI 300086 into pUC18 and transforming those recombinant plasmids into Escherichia coli (Smith et al., 1987).

RESULTS AND DISCUSSION

Experiment 1:

Persistence and forage yields for the five years is shown in Figure 1. In the first year of establishment, yields were low, with only PI 300086 showing a significantly higher yield than the other entries in 1985. The tetraploid varieties, 300086, N-128

and Mott clearly outyielded the four triploid hybrids the other years. The three hybrids, S4, S37, and S38 were similar in yields, morphology and other characteristics and were pooled. One possible reason for the low persistence and lower yields of the triploid hybrids is the fact that one of the parents (pearl millet) is an annual. The F_1 hybrids of the annual x perennial cross evidently give weaker perenniality in the hybrids than in the tetraploid parental lines.

Figure 2 shows the NDF percent values each year for the seven lines tested. Again, three of the F_1 triploids were pooled. Lines that yielded the highest dry matter (See Figure 1) also had corresponding high fiber content. The mean NDF percent for each variety over the five years was 80.3, 79.6, 76.1, 74.5 and 74.6 for 300086, N-128, Mott, three pooled triploids (S4, S37, S38), and S3, respectively.

High CP and IVOMD are desirable attributes and it was in these two characteristics that the triploid hybrids showed superiority (See Figures 3 & 4). PI 300086 and N-128 were consistently and significantly lower in CP and IVOMD each year of the five-year test. This is due to the much higher leaf/stem ratio in the two tall growing tetraploids when compared to the dwarf variety, Mott and other lines. The protein percent of PI 300086 and N-128 is so low that animals cannot meet their requirement for maintenance. If the CP is multiplied by dry matter yield in Mg/ha to give a quality component to the forage, the values would be about 0.80, 0.70, 0.66, and 0.40 for 300086, N-128, Mott and S3, respectively. Again the three triploid hybrids failed to show any superiority over cultivar Mott. High dry matter yield is undesirable if the quality of the grass is so low that animals cannot be maintained on it. The 4 and 5 percent CP values make PI 300086 and N-128 totally unacceptable as cattle feed if only harvested one or two times per year. Only Mott and the triploid F_1 hybrids have sufficiently high CP values for acceptance. The persistence of Mott has proven better, and since the yield of it is three times higher that the triploid hybrids, we cannot recommend the hybrids over Mott until further testing.

Experiment 2:

In 1988, three of the replications were not harvested for yield, but were used for propagation of other experiments. The remaining three replications were harvested giving means of 9.1, 11.8 and 11.8 Mg/ha for Mott, three pooled triploids and three pooled hexaploids, respectively. In IVOMD analyses in 1988, 506 was significantly better than 362 (both are hexaploids). None of the hybrids at either the triploid or hexaploid level were significantly different than Mott. No differences were found in CP or NDF during 1988.

FIG. 1. DRY MATTER FORAGE YIELDS - 1985-1989

PENNISETUM LINES AT BEEF RESEARCH UNIT

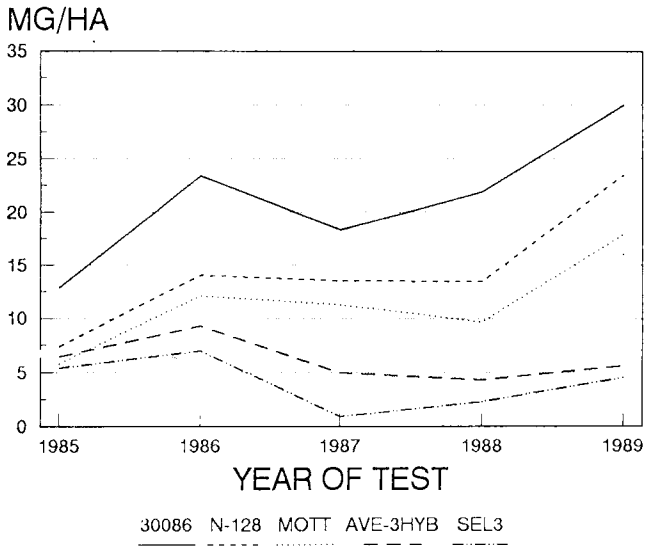


FIG. 2. NEUTRAL DETERGENT FIBER (NDF) 1985-1989

IN PENNISETUM LINES AT THE BEEF RESEARCH UNIT

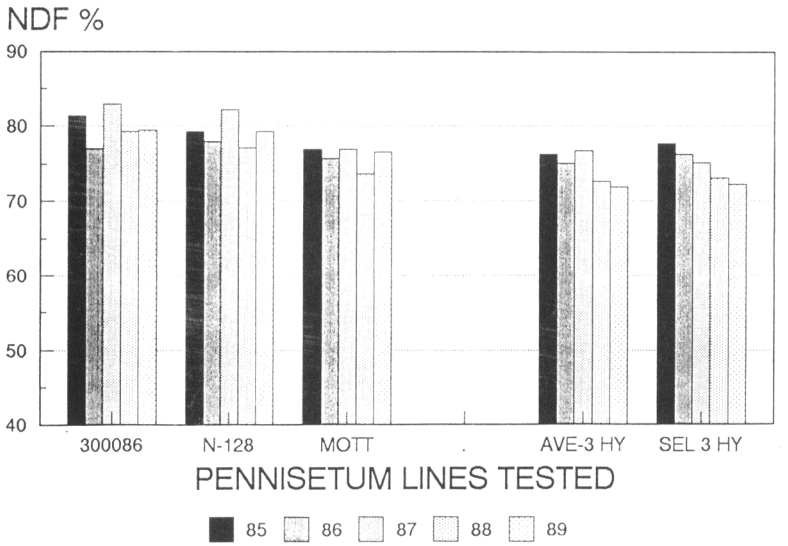


FIG. 3. CRUDE PROTEIN (CP) FIVE YEAR AVERAGE
IN PENNISETUM LINES AT THE BEEF RESEARCH UNIT

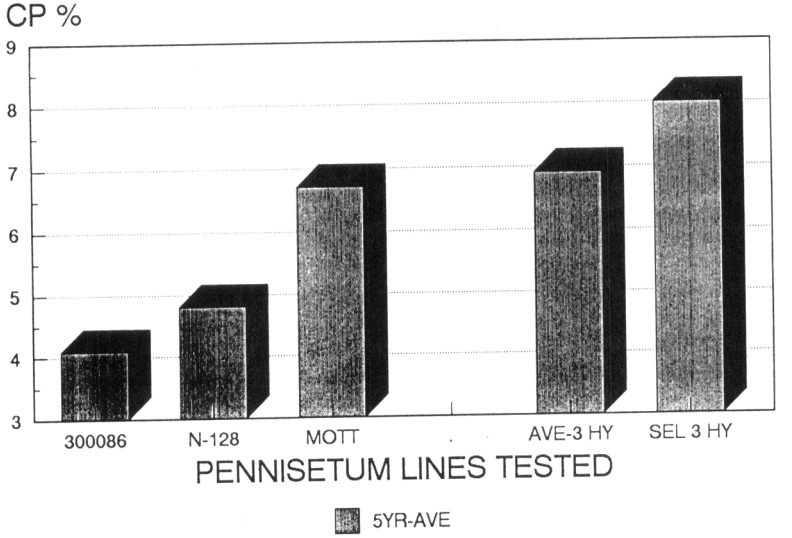
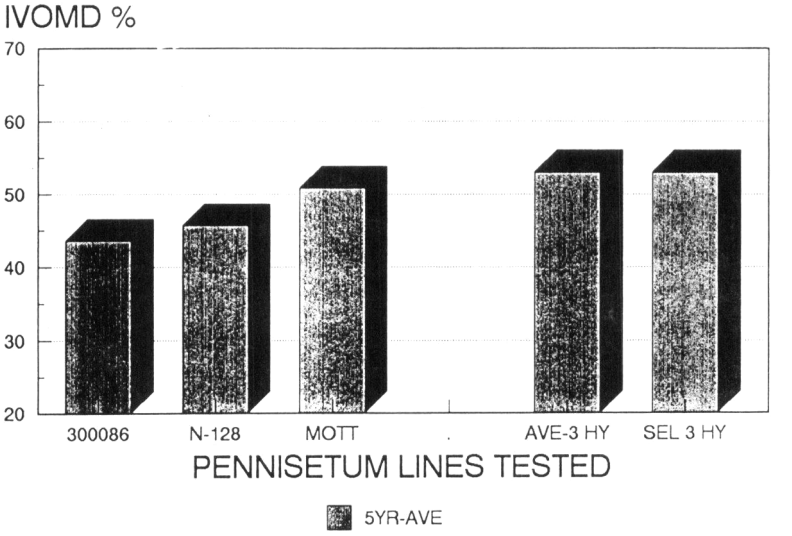


FIG. 4. IN VITRO DIGESTIBILITY (IVOMD)
IN PENNISETUM LINES AT THE BEEF RESEARCH UNIT



Yields in 1989 (Figure 5) were dramatically different from the previous year. Mott gave the highest (10 Mg/ha) whereas the triploids averaged 5.7 and the hexaploids 5.9 Mg/ha. The reduction in yield of the triploids and hexaploids was caused by a much lower winter survival. Mott had an average of 100 percent survival in each replication of experiment 2, whereas the triploids had an average of 53 and the hexaploids had 54 percent survivors.

None of the triploids or hexaploids were significantly better than Mott in NDF values. However, in IVOMD, Figure 6, S41 S4 and 506 were significantly better than Mott in the November harvest. CP values are shown in Figure 7. S3 was significantly better than Mott in July and both S3 and 506 were significantly better in November. When protein content is multiplied by yield to get a combined protein yield value, Mott is superior over an of the triploids or hexaploids. The comparative values were 1.0 0.76, and 0.66 for Mott, S41 and 506.

Development and Use of RFLP Markers in Pennisetum: Napiergrass and its interspecific hybrid with pearl millet possess a great deal of restriction fragment polymorphism as nearly all non-repetitive random genomic probes tested have sho polymorphism when tested on DNA from a genetically diverse test set or the plant introduction collection. As RFLP markers are developed they are being mapped into linkage groups using data from segregating interspecific progenies analyzed by the comput program, 'Linkage-1' by Suiter et al. (1983). Figure 8 shows th segregation of two polymorphic markers in an interspecific progeny (1 to 17) along with the parents of the interspecific cross, pearl millet, Tift 23DA (designated A1) x napiergrass, Mott (designated 75). RFLP markers are also being associated wi plant trait genes by linkage. To date, preliminary data have shown RFLP markers associated with leaf length, leaf width, pla height, stem weight and plant pubescence. Markers linked to oth plant trait genes, especially those of the plant composition ar forage quality are being sought. Markers linked to plant trait genes, especially those of quantitative traits, can be very helpful in the genetic manipulation of those traits.

We have RFLP markers available that are capable of identifying each of the genotypes in our napiergrass collectior and have used those markers to measure the genetic diversity of those lines.

CONCLUSIONS

In the comparisons (experiment 1) between Mott and tall tetraploid varieties, although Mott was lower in Mg/ha, the higher CP % made Mott more acceptable than any other line test in the five-year experiment.

FIG. 5. THREE TRIPLOID & THREE HEXAPLOID HYBRIDS

COMPARED TO DWARF MOTT ELEPHANTGRASS AT BRU 1989

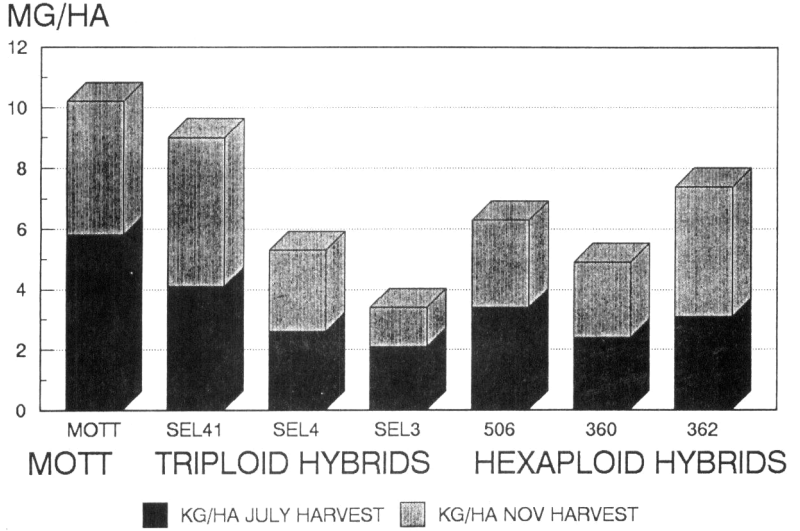


FIG. 6. IN VITRO DIGESTIBILITY (IVOMD)--HYBRIDS

COMPARED TO DWARF MOTT ELEPHANTGRASS AT BRU 1989

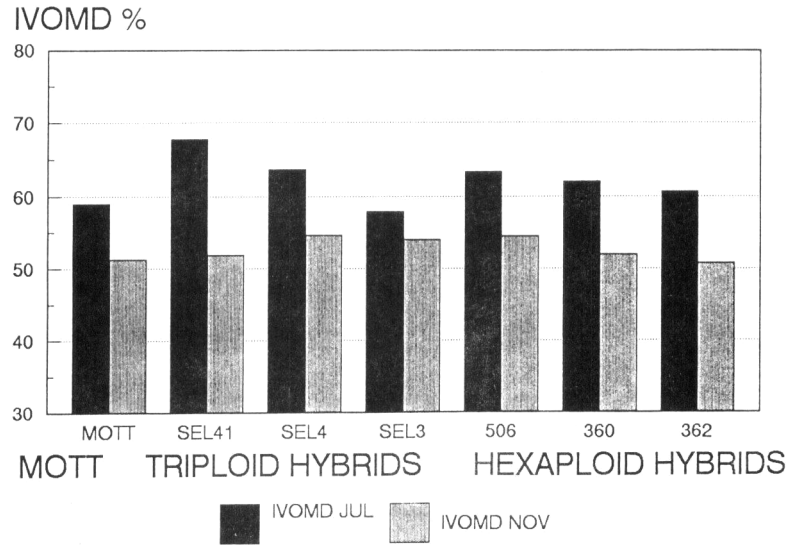
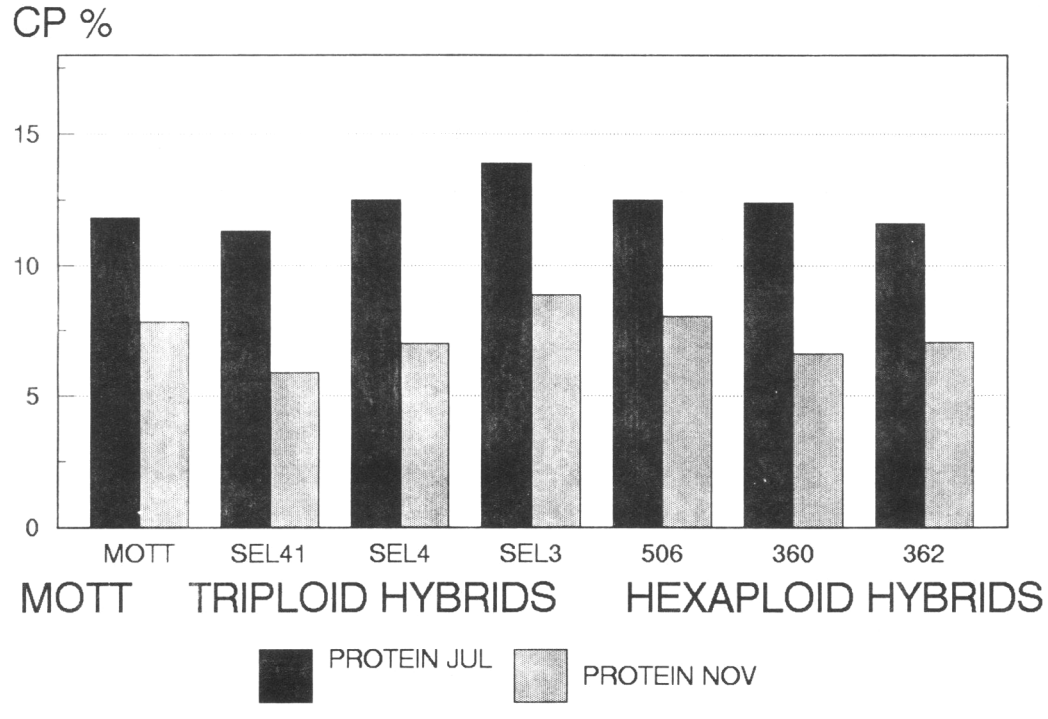


FIG. 7. CRUDE PROTEIN (CP) TRIPLOID & HEXAPLOIDS

COMPARED TO DWARF MOTT ELEPHANTGRASS AT BRU 1989



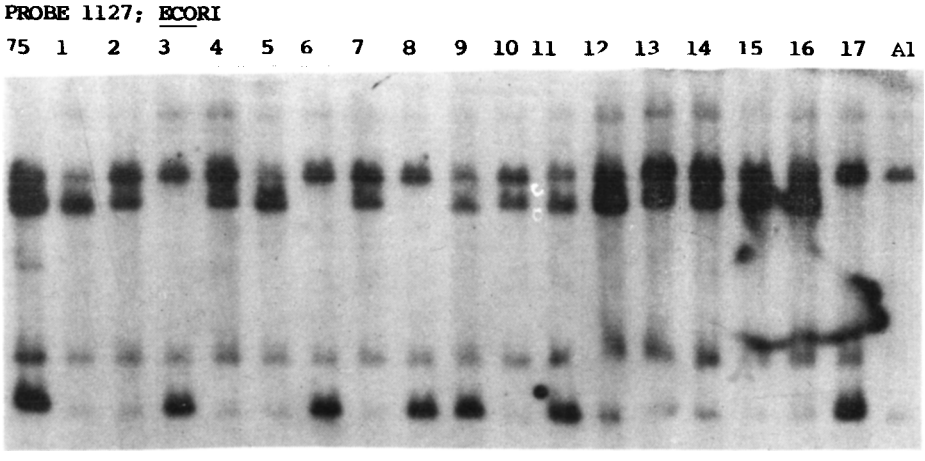


Fig. 8. This autoradiograph illustrates the RFLP segregation of the triploid progeny of the interspecific hybrid Tift 239DB X Mott. The restriction fragments of the pearl millet parent (A1) is found in each of the progeny and the variation of restriction fragments occurs in the fragments obtained from the napiergrass parent Mott, listed as 75 on the figure. Progeny are numbered 1 to 17.

In experiment 2, Mott again showed superiority in winter survival, dry matter yield, and had sufficiently high CP and IVOMD to be accepted as a livestock feed. Although some of the triploids and hexaploid derivatives were slightly higher in CP, the disadvantages of low winter survival and subsequent low dry matter yields in 1989 offset the higher CP values. At this time we cannot recommend the new hybrids over Mott in an environment in which winter survival may be a factor.

The RFLP experiments have shown that molecular genetic methodology can be applied to the genus Pennisetum. The napiergrass genome was found to be highly polymorphic in RFLPs. The usefulness of using RFLP markers to identify hybrids between species, and within a species has been established.

ACKNOWLEDGMENTS

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