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DEVELOPMENT OF *PANICUM SP.* HYBRIDS USING APOMICTIC PLANTS

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ABSTRACT

Guineagrass (*Panicum maximum* Jacq.) is an excellent forage grass. One of its major weaknesses is its seed shattering and low seed yield. Efforts to increase seed retention in guinea-grass have been unsuccessful due to the lack of genetic material having this characteristic and also to the difficulty encountered in the hybridization process. The reproduction of guineagrass is based on aposporous apomixis with pseudogamy. For the development of *Panicum* hybrids, we utilized facultative apomictics and two germplasm sources previously discovered. A possible hybrid was obtained using facultative apomictics PI 277901 and CIAT 673. Utilizing RAPD analyses, the following hybrids (2n=32) were verified: SPM-92 x PRPI 3622 and Tift-49 x PRPI 3622. Natural and hand-made crosses of paragrass (*P. purpurascens* Raddi) x klein-grass (*P. coloratum* L.) (2n=36) were also verified utilizing RAPD analyses. This study reports for the first time the existence of new Panicum hybrids including an interspecific hybrid of para x klein grass and the utilization of RAPD's as an excellent tool for the verification of the existence of hybrids in the Panicums.

INTRODUCTION

Probably the most important representative of the Panicums is guineagrass (*Panicum maximum* Jacq.), a warm-season perennial bunchgrass. Besides being adapted to many ecological conditions in the tropics and subtropics the high yielding potential of this forage is well known. Guineagrass reproduces by aposporous apomixis with pseudogamy and the progenies are genetically similar to those of the mother plants although sexual plants have been discovered in populations of several introductions (Burton, et al. 1973). The method of reproduction of guineagrass and other related apomictic species might explain the absence of hybrids that have been reported in the Panicums.

In Florida, Smith (1972) discovered the existence of sexual plants in *P. maximum* populations of PI 156542, 277901 and 277962 while in Georgia, Burton et al. (1973) discovered two sexual plants in facultative apomictic introductions, PI 277946 and 277922. These five different cytoplasms could probably offer alternatives for the use in the development of hybrids or to reduce the genetic vulnerability of the genus. In Georgia, Hanna (1993) developed and released a sexual clone of *P. maximum* (Tifton SPM92) which has great potential in a breeding program.

One of the objectives of our breeding program is to reduce seed shattering in guineagrass (2n=32) which could be accomplished by transferring seed retention from a related species, *Panicum coloratum* L. (kleingrass) (2n=36). Since the two species differ in chromosome number, we do not expect them to cross through regular breeding methods. An additional alternative that we are exploring is the evaluation of advanced generations of crosses between sexual x apomictic plants, which might release the genes for resistance to shattering.

Another related species of guineagrass is *P. purpurascens* Raddi, known as paragrass or "malojillo." It is a native of Africa, from where it was introduced into Brazil (Hitchcock, 1935). Paragrass is a vigorous, stoloniferous species with stems reaching a length of 1.8 to 4.7 meters, and generally rooting at the nodes. It is widely found in moist and poorly drained soils, and if managed properly can produce abundant forage (Alberts and García-Molinari, 1943). Kleingrass and paragrass, having the same chromosome number (2n=36), offer an excellent opportunity to study the inheritance of

seed shattering present in kleingrass. This study reports basic cytology work of some breeding accessions of *P. maximum* and the characterization of parents and hybrids using cytogenetic and molecular techniques.

MATERIALS AND METHODS

The plants used in this study as female parents were Tifton *Panicum maximum* 49 (Tift PM49), Tifton sexual *Panicum maximum* 92 (Tift SPM92), Borinquen, and a series of lines previously identified as potential sexual plants discovered in facultative apomictics by Burton et al. (1973) and Smith (1972). As compared to the common guineagrass, cultivar Borinquen is a more delicate grass than other Panicums and contains a high leaf to stem ratio. The Borinquen plant material used in the breeding program was collected by the senior author in the city of Mayaguez near highway number 2.

During 1993 crosses were made in the greenhouse at TARS, Mayaguez, Puerto Rico and under field conditions at the Isabela ARS farm. Panicles of the female parents approaching flowering, were covered with plastic bags in late afternoon and removed next morning at sunrise. Exserted stigmas between the non-dehiscing anthers were dusted with pollen from the male parents and panicles were then enclosed in brown paper bags. Seed of each cross was sown on jiffy-pots containing a mixture of soil and filtered press-cake. The germinated seedlings were individually transplanted to 20 cm pots. The progeny was morphologically evaluated and biochemically analyzed to identify the presence of hybrids.

Pollen mother cells and root-tips were used to determine chromosome number. The first procedure, (Burson and Bennett, 1970), consisted of collecting and fixing immature inflorescenses in Carnoy's solution (6:3:1) to study microsporogenesis. Pollen mother cells were stained with aceto-carmine and examined using phase contrast microscopy. In the second procedure, (Hanna et al., 1973), root tips were collected in the morning (7:00-9:00), and placed in 1-bromonaphthalene for two hours. After hydrolysis in 1N HCl for 10 minutes at 60° C they were stained using Fuelgen stain, macerated on acetocarmine and observed using phase contrast microscopy.

To characterize the parents and hybrids used, DNA was extracted using the "mini-prep" procedure of Afanador et al. (1993), with modifications. In this procedure, DNA is extracted from 2"- 4" sections of the immature rolled leaf base. Six hundred microliters of extraction buffer were added to the leaf tissue in a mortar and 100 to 150 mg of sterile sand used to grind the leaf tissue. Further procedures were those used for bean DNA analyses (Afanador et al.,1993). Extracted DNA was examined using RAPD analyses (Haley et al., 1993).

RESULTS AND DISCUSSION

The examination of root tips and pollen mother cells of sixteen Panicums from the TARS collection showed a chromosome number of 2n=32 for *P. maximum* and 2n=36 for *P. coloratum* (PI 410177), *P. purpurascens* and *P. purpurascens* x *P. coloratum* (Table 1).

The progeny derived from the crosses between: a. *P. maximum* (Borinquen) x *P. coloratum* (Kleingrass) and b. *P. maximum* (PI 277901) x *P. maximum* (CIAT 673) was morphologically identical to the female parent, suggesting that self-pollination had occurred. The three other crosses made: a. *P. maximum* (SPM-92) x *P. maximum* (PI 3622), b. *P. maximum* (Tift 49) x *P. maximum* (PI 3622) and c. the interspecific cross between *P. purpurascens* (Paragrass) x *P. coloratum* (Kleingrass), produced hybrid progeny (Table 2).

The patterns generated by decamer primers of random sequence, were examined for polymorphic RAPD's between the parents that are recombined in the hybrids. Primer OAN-17 identified Tift-49 x PI 3622, SPM-92 x PI 3622, and *P. purpurascens* x *P. coloratum* (Figs. 1,2), as hybrids.

These results demonstrate the feasibilty of using RAPD's analysis as a tool for identifying hybrid progeny, in the efforts directed at solving the seed shattering problem. Recovery of an interspecific

progeny, in the efforts directed at solving the seed shattering problem. Recovery of an interspecific hybrid from the *P. purpurascens* x *P. coloratum* cross demonstrates the possibility of transferring the genes involved in seed shattering. This would permit their manipulation for the reduction or elimination of a great weakness of *Panicum maximum*.

Results from preliminary evaluations of this hybrid still indicate a tendency to seed shattering. Further crossing and evaluations are required to assess the nature of the inheritance of this trait.

CONCLUSIONS

The identification of guineagrass hybrids from crosses involving sexual and apomictic plants, increases the possibilities for the release of the genes for shatter resistance. Recovery of an interspecific hybrid from the *P. purpurascens* x *P. coloratum* cross confirms the possibility of manipulating and studying the genes involved in seed shattering. The use of RAPD's analyses as a tool for the identification of hybrid progenies will make an important contribution in the efforts to improve seed retention in guineagrass.

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Table 1. Chromosome number in sixteen selected *Panicum* accessions from TARS collection.

Plant Species and Introduction Number	Chromosome Number (2n)
1. *P. maximum* (PI 3622)	32
2. *P. maximum* (PI 156542)	32
3. *P. maximum* (PI 277901)	32
4. *P. maximum* (PI 277922)	32
5. *P. maximum* (Borinquen)	32
6. *P. maximum* (Tift PM49)[1/]	32
7. *P. maximum* (Tift SPM92)[2/]	32
8. *P. maximum* (CIAT 673)[3/]	32
9. *P. maximum* (CIAT 6171)	32
10. *P. maximum* (CIAT 6180)	32
11. *P. maximum* (CIAT 6501)	32
12. *P. maximum* (CIAT 6533)	32
13. *P. maximum* (CIAT 6567)	32
14. *P. maximum* (PI 410177)	36
15. *P. purpurascens*	36
16. P. purpurascens x P. coloratum	36

[1/] Tifton *P. maximum* 49
[2/] Tifton sexual *P. maximum* 92
[3/]Centro Internacional de Agricultura Tropical, Cali, Colombia

Table 2. *Panicum spp.* utilized as parental material.

Female Parent	Male Parent
P. maximum (Borinquen)	*P. coloratum* (Klein)
P. maximum (Tift SPM92)	*P. maximum* (PI 3622)
P. maximum (Tift PM49)	*P. maximum* (PI 3622)
P. maximum (PI 277901)	*P. maximum* (CIAT 673)
P. purpurascens (Pará)	*P. coloratum* (Klein)

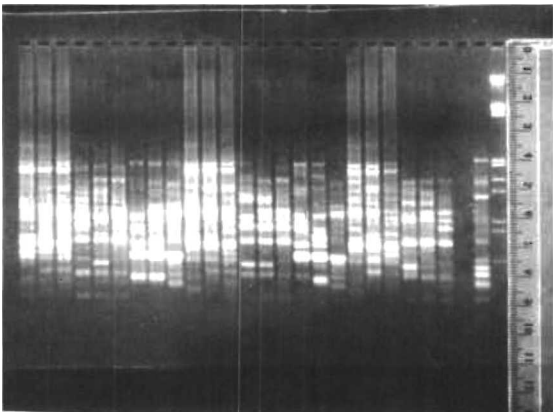

Fig. 1. Ethidium bromide stained PCR products amplified by decamer primers and separated on 1% agarose gel.

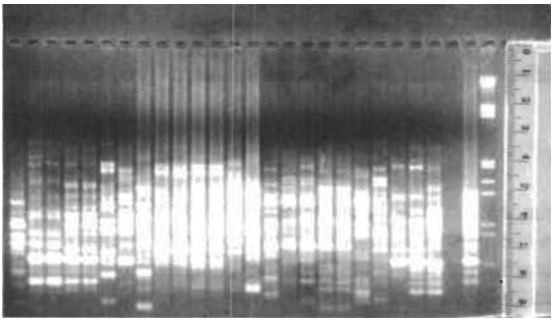

Fig. 2. Ethidium bromide stained PCR products amplified by decamer primers and separated on 1% agarose gel.