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BREEDING FOR SEED SHATTERING RESISTANCE IN GUINEAGRASS

A. SOTOMAYOR-RIOS, E. RIVERA-AMADOR, and A. QUILES-BELEN

USDA-ARS, Tropical Agriculture Research Station
Mayaguez, Puerto Rico

INTRODUCTION

The genus *Panicum* comprises some 500 grass species distributed throughout the tropical and warm-temperate regions of the world (HITCHCOCK, 1935). These include annual and perennial forms, many of which are of economical importance. A prime example is guineagrass (*P. maximum* Jacq.), which has been widely recognized as an excellent perennial forage for grazing, hay, and silage due to its high yields and adaptation to almost all soil types and environmental conditions in the tropics. WARMKEE (1951), distinguished five strains of guineagrass in Puerto Rico, and designated them as Local or Common, Gramalote, Borinquen, Broadleaf, and Fineleaf on the basis of agronomic and morphological traits. Of these, Local or Common Gramalote (2n=32) is the most widely utilized on the island. However, two of the major constraints of guineagrass are its seed shattering and its indeterminate flowering behavior, resulting in low and poor quality seed production. Cytological studies on four guineagrass varieties (WARMKEE, 1954) four decades ago showed that this species is a facultative apomictic, having both apospory (derivation of an unreduced gametophyte from somatic cells of the nucellus or chalaza) and pseudogamy (pollination is essential for seed development although fertilization of the egg does not take place). WARMKEE concluded, based on progeny tests, that the off-type plants he obtained (1.3 - 4.7 percent) had arisen from sexual recombination. Smith (1972) isolated completely sexual plants of *P. maximum* by testing off-type plants in species formerly believed to be apomictic. HUTTON (1989) at EMBRAPA, Brazil, reported the development of promising acid-

tolerant lines of *P. maximum*. Through embryo sac analysis Javier (1970) reported the sexual potential in six guineagrass varieties ranging from 22 to 53 percent, although no sexual plants were isolated by this author. Obligate apomixis is genetically controlled (BASHAW, 1962) and can be manipulated. According to Burton et al. (1973), "to release the variability in obligate apomictic plants, they must be hybridized with a compatible sexual plant used as the female parent." In order to improve any obligate apomict genetically, sexual compatible plants must be discovered. Possibilities exist to improve seed shattering in guineagrass by interspecific hybridization with shatter-resistant *Panicums* such as kleingrass (*P. coloratum* (L.)), or *P. fasciculatum* Sw. (2n=36), although efforts to increase seed retention in guineagrass through breeding have been unsuccessful. This paper outlines our progress in attempting to decrease seed shattering in guineagrass through breeding and in using isoenzyme analysis to assist in the determination of the genetic diversity of the *P. maximum* lines and hybrids.

MATERIALS AND METHODS

In 1990, seeds of kleingrass, (PI 410177) (2n=36), provided by Dr. B. A. YOUNG, USDA-ARS, Temple, Texas, were introduced to the Tropical Agriculture Research Station. This PI is the only known seed-shattering resistance source of *P. coloratum* (YOUNG, 1986). Two sexual guineagrass sources, Tift PM49 (Tifton 49, *P. maximum*) described by HANNA *et al.* (1973) , a clone producing sexual and apomictic plants, and seed of Tift SPM92 (Tifton sexual *P. maximum*) from Dr. W. HANNA's USDA-ARS, Tifton, Georgia, research program (HANNA, 1993) were also introduced into TARS. The two sexual guineagrass sources were used as the female in crosses with a series of guineagrass cultivars (Table 1), and kleingrass. Crosses were made in the greenhouse during 1992 at TARS, Mayaguez and under field conditions at the Isabela ARS farm, Isabela, Puerto Rico. Seven *P.maximum* selections and sexual Tift PM49 and SPM92 (female parents) were used in the crossing program. Panicles of the female parents approaching flowering were covered with plastic bags in the late afternoon, which were removed next morning at sunrise. Exserted stigmas between the nondehiscing anthers were dusted with pollen from

the male parents and panicles were then enclosed in brown paper bags. Seeds of each of the crosses were surface sown on jiffy-pots containing a mixture of soil and filtered press-cake and then transplanted singly to small 20 cm pots. Embryo sac analyses were conducted by collecting inflorescenses when fully emerged or at the beginning of stigma exsertion (YOUNG *et al.*, 1979). Spikes were fixed in formalin-acetic-acid-alcohol (FAA) (90 ml 70% ethanol, 5 ml acetic acid, and 5 ml formaldehyde) for 24 hrs. Spikes were then immersed for a 30-minute period in each of three concentrations of ethyl alcohol (50, 70, and 95%) and for two 30-minute periods in absolute ethyl alcohol. This stage was followed by one-hour immersions in each of three combinations of a methyl salicylate /absolute ethyl alcohol mixture: (50/50, 75/25, 85/15%) and up to 48 hours in 100% methyl salicylate. Ovaries were dissected, placed on a glass slide having a few drops of methyl salicylate and observed under a phase contrast microscope. The spatial arrangement and relative size of the embryo sac components served as parameters in the determination of the mode of reproduction of the Panicums studied.

To support the morphological data, isozyme variabilities were observed. The method utilized for the isozyme analysis was as follows: Proteins were extracted by grinding 2.0 g of leaf tissue with a cold mortar and pestle in 5.0 ml of 0.05 M Tris-HCl (pH 7.5) and 100 ul of B-mercaptoethanol. Gels were prepared using 48.0 g of Sigma starch, 14.0 g of sucrose, and 400 ml of gel buffer. Three gel and electrode buffer systems were used: histidine-citrate (pH 7.5) for malate dehydrogenase (MDH), isocitrate dehydrogenase (IDH), and Phosphoglucomutase (PGM); morpholine-citrate (pH 6.1) for glutamate dehydrogenase (GDM), Shikimate Dehydrogenase (SKD), and Peroxidase (PRX); tris-borate-EDTA (pH 8.6) for phosphoglucoisomerase (PGI) and glucose-6-phosphate dehydrogenase (G6PD). The enzyme staining systems of SOLTIS and SOLTIS (1989) were used with slight modifications. Of these enzymes, MDH and G6PD were selected for the final analysis because they showed differences in loci between the samples, and overall electrophoretogram resolution was consistent.

RESULTS AND DISCUSSION

Two procedures which we could utilize to obtain genes for shatter resistance in *P. maximum* are: (1) transfer shatter resistance from other *Panicum* species such as kleingrass by interspecific hybridization and (2) screen advanced generations of sexual x apomictic hybrids. We have utilized two facultative apomictic introductions (PI 277946 and 277922) from which sexual plants were discovered by BURTON *et al.* (1973) and which gave fertile hybrids when crossed with giant short-day types. Also, three facultative apomictic introductions (PI 156542, 277901 and 277962) from populations in which SMITH (1972) had discovered sexual plants are being utilized. Sexual clones (Tift 49) and seed of Tift SPM92 supplied by HANNA (1993) have been of value in our work to date. We are examining possible "hybrids" between Tift SPM92 x PR PI 3622 (USDA PI 259553), one of our best male parents. Plants produced by this cross inherit a dominant leaf constriction of the male parent, PI 3622. Other traits under study such as stem pigmentation, hairiness, and growth habits might prove effective in locating off-type plants. Data on number of plants obtained from crosses of various male and female parents are given on Table 1.

Embryo sac analyses of several *Panicum* species (Table 2) resulted in the preliminary identification of sexual plants in PI 3622, which was believed to be an obligate apomictic. These results, where a new *Panicum* has been identified as facultative apomictic and added to the existing group, would increase the probabilities of obtaining the genes for shatter resistance through intraspecific and interspecific hybridization. New analyses are still being conducted since new additions have been recently made to the *Panicum* collection at TARS.

Results of the isozyme work being conducted are still inconclusive and will be reported when the analyses are completed.

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Table 1. Plants of guineagrass obtained from crosses between *P. maximum* x *P. maximum* and *P. maximum* x *P. coloratum*, Mayaguez, Puerto Rico.

Parent Plants		Plants
Females	Males	obtained
Tift SPM92[1/]	CIAT[3/]	1
Tift SPM92	259553	3
Tift PM49	CIAT 604	5
Tift PM49[2/]	259553	5
Tift SPM92	*P. coloratum* (PI 410177)	4
PI 277962	CIAT 604	20
PI 277962	CIAT 673	21
PI 277946	CIAT 604	35
PI 277946	CIAT 673	41
PI 277922	CIAT 673	43
PI 277922	CIAT 604	3
PI 156542	*P. coloratum* (PI 410177)	30

[1/] Tifton sexual *Panicum maximum* 92

[2/] Tifton *Panicum maximum* 49

[3/] Centro Internacional de Agricultura Tropical, Cali, Colombia

Table 2. Mode of reproduction of selected *Panicums* as determined by embryo sac analysis.

Apomictic	Sexual	Facultative
P. coloratum	Tift SPM92	Tift PM49
CIAT 673		PI 3622
		PI 277946
		PI 277901
		PI 277962
		PI 156542
		PI 277922