Reprinted from *Crop Science*
Vol. 22, May-June 1982, p. 467-469

Embryological Analysis of Facultative Apomixis in *Panicum maximum* Jacq.[1]

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ABSTRACT

Eighty apomictic accessions of Guineagrass (*Panicum maximum* Jacq.) collected mainly in East Africa, and 80 apomictic hybrids from sexual × apomictic crosses, were studied for their mode of reproduction. The objective was to determine to what extent percentage sexuality in facultative apomixis can be modified following hybridization. Apomictic accessions with a high percentage sexual reproduction were observed among biotypes from South Africa and the Antilles. Three of the hybrids also exhibited a high percentage of sexuality, while almost all the others appeared to be obligate or nearly obligate apomicts. The average percentage sexual reproduction observed in apomictic hybrids was 5.6 compared to 8.1 in apomictic accessions. Thus hybridization did not increase sexuality in apomicts of *P. maximum*.

Embryological analyses appeared to be especially efficient and a rapid means for determining the mode of reproduction in *P. maximum*. However, because apomixis is essentially obligate or nearly obligate in this species, progeny tests are no less reliable than embryological analyses.

Based on these and previous findings, there is no doubt that apomixis, although facultative, can be easily manipulated in *P. maximum*.

Additional index words: **Guineagrass, Sexuality, Embryo sacanalysis, Progeny tests, Variability**

Panicum maximum Jacq., although called Guineagrass, is native to East Africa, Kenya, and Tanzania. It is the main forage grass used in Queensland, Australia, and is grown on several million hectares in Central and South America.

Biology of *P. maximum* has been extensively studied by Warmke (1954) and Combes (1972). Most *P. maximum* biotypes are tetraploids which reproduce by facultative apomixis.

Obligate sexuality was first discovered in small diploid populations from Tanzania by Combes and Pernès (1970). Nakajima et al. (1979) reported the discovery of new diploid biotypes, recently introduced from the same country. Meanwhile sexuality was found at the tetraploid level, in progenies of apomictic accessions from South Africa, by Smith (1972) and Hanna et al. (1973).

Crossing experiments were then attempted, using sexual tetraploids as pistillate, and apomictic tetraploids as staminate parents. The sexual tetraploids were obtained from colchicine-treated natural sexual diploids. The crosses gave progenies including both sexual and apomictic hybrids (Combes, 1972; Pernès, and René-Chaume, 1973). Among 59 hybrids obtained by Hanna et al. (1973), embryological observations indicated that 21 were sexual and 28 were apomictic. The hypothesis of a one to one ratio they presented has since been confirmed by Savidan (1980). Furthermore, the segregations observed in 10 different cross combinations clearly demonstrated that apomixis in *P. maximum* is dominant over sexuality and determined by no more than a single gene (Savidan 1981a, 1981b). The genotype of sexual tetraploids was defined as aaaa, while all the apomicts tested showed the same Aaaa genotype.

Apomixis results from the combination of two components, failure of reduction and failure of fertilization. These two appear to be linked in our material, and inherited with the A gene. However, in addition apomixis in *P. maximum* is often facultative. Thus, a low percentage of sexual reproduction, the "sexual potential", can be observed as ovules with a single 8-nucleate embryo sac, or off-types within the progeny. In a study of reproductive system in 295 plants of *P. maximum,* based on a 10 to 20-plant progeny test, Burton et al. (1973) classified 259 plants (87.8%) as obligate apomicts. In general, embryological analyses reveal a higher sexual potential (Warmke, 1954; Bogdan, 1963; Combes, 1972; Pernès et al., 1975). Regardless of whether a morphological or an embryological analysis is used to measure the percentage sexual reproduction, the problem is to determine if hybridization results in a large increase in this percentage. As Bashaw (1974) stated, "facultative apomixis has very limited potential for use as a breeding tool unless highly apomictic lines can be recovered." The objectives of this study were to 1) analyze the embryological variation among 80 apomictic accessions chosen at random in our field collection, 2) observe whether the variation could be different in a sample of 80 F_1 or three-way apomictic hybrids, and 3) discuss the value of cytological estimates of a percentage sexual reproduction, and the potential for use of the *P. maximum* apomictic hybrids.

MATERIALS AND METHODS

A nursery of 500 accessions was established in April 1969, following two exploration trips to Kenya and Tanzania. Eighty tetraploid apomicts were randomly chosen from this collection and their percentage sexual reproduction was determined. They were compared with 80 apomictic hybrids which were part of materials presented in a previous paper (Savidan, 1980). S2.T was a sexual tetraploid obtained from the colchicine treatment of a diploid, while IS3 and IS4 were two sexual F_1 hybrids from the cross K189.T × G3. Approximately 100 ovules were studied from each plant. Results of examination of about 15,700 ovules are reported in this paper.

Apomixis in *P. maximum* is associated with ovules that contain multiple embryo sacs, or ovules that have a single 4-nucleate embryo sac. Because the sexual sacs that are observed in ovules with multiple embryo sacs are rapidly crushed by competition, the percentage sexual reproduction reported is the percentage ovules with a single 8-nucleate embryo sac (Savidan, 1978, 1980).

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Table 1. Embryological analysis of 80 apomictic accessions of *P. maximum.*

Area of origin	No accessions	Sexual reproduction	
		Mean	Range
		%	
Kenya	34	4.0	0-18
Tanzania	26	8.9	0-67
South Africa	9	12.2	0-53
Other African countries	5	7.0	0.8-22.6
Brazil	2	2	0-4
Antilles	4	33.5	20-40
Total	80	8.1	0-67

In the terminology used in these *P. maximum* studies, a sexual plant is a plant which only shows 8-nucleate embryo sacs as single sacs and gives heterogeneous progenies. An apomictic plant is characterized by the 4-nucleate embryo sac, although it could show several types of ovules, and even ovules with a single 8-nucleate embryo sac.

Chromosome numbers were determined from inflorescences of all plants fixed in Carnoy (60% absolute alcohol, 30% chloroform and 10% acetic acid) and stained in hematoxyline. Embryo sac analyses were conducted using the clearing squash technique described by Herr (1971).

RESULTS AND DISCUSSION

Apomictic Accessions

Ten apomictic accessions showed no sexual ovule development. The great majority of the others would appear to be obligate from a 10 to 20-plant progeny test. Variability was observed among the South African accessions(Table 1). For instance, in accession G69 apomixis was obligate, while 53% of the ovules in accession G13 had a single sexual embryo sac. Antilles may be a secondary area of diversification for *P. maximum* because accessions G90, G100, 102, and 104 exhibited 20, 39, 40, and 35% ovules with a single sexual embryo sac, respectively. The highest percentage sexual reproduction was observed in Accession T19, from Tanzania, which appeared to be of interspecific origin as many morphological characters were intermediate between *P. maximum* and *P. infestum* Anders.

Obligate sexual tetraploids could be recovered in progenies from apomicts that show a high level of sexual reproduction, as in some South African accessions. Apomicts of *P. maximum* have a Aaaa genotype (Savidan, 1981a, 1981b), in which A conditions apomixis. In progenies from apomicts, one-fourth of the off-types thus theoretically get a sexual aaaa genotype. In analyzing 68 off-types from T19, we found 50 apomicts and 18 plants which showed only 8-nucleate embryo sacs as single sacs (832 ovules observed). This could account for the sexual tetraploids discovered by Smith (1972) and Hanna et al. (1973).

Apomictic Hybrids

Twenty-one hybrids appeared to be obligate apomicts. Forty-two others showed less than 5% sexual embryo sac development. Three hybrids exhibited a high sexual potential. The female parent of all three hybrids was IS4. More variation for reproductive behavior was observed in

Table 2. Embryological analysis of 80 apomictic hybrids between sexual and apomictic *P. maximum.*

Cross combinations	No hybrids	Sexual reproduction	
		Mean	Range
		%	
S2.T × apo	23	1.2	0-4
IS3 × apo	25	3.4	0-31.7
IS4 × apo	32	10.3	0-88
Total	80	5.6	0-88

three-way hybrids (hybrids from IS3 and IS4 in Table 2) than from F_1's (hybrids from S2.T). All hybrids using S2.T as the female parent were even obligate or nearly obligate apomicts.

The average percentage sexual reproduction observed in the apomictic hybrids was 5.6 compared to 8.1 in the original apomictic accessions. Thus, though rare exceptions were observed, hybridization did not increase the sexual potential in facultative apomicts. There is no doubt that apomixis, although facultative, can be easily manipulated in *P. maximum.*

Cytological Estimates vs Progeny Tests

Embryological analyses are often considered inconclusive by plant breeders, while geneticists do not agree with exclusive use of progeny tests. However, their objectives are sometimes different.

If the purpose is to screen obligate apomicts from obligate sexuals in a given hybrid population, progeny tests can be used. In the case of *P. maximum,* the embryological test is no less reliable than the progeny test. Furthermore, it is more rapid than the progeny test because the former is applied to the plant itself while the latter is made on the progeny, i.e., one generation later.

Results of progeny tests may not be reliable when apomixis is facultative, with high percentage sexual reproduction. The present analysis showed that is an exceptional situation in *P. maximum.*

If the purpose is to know only the degree of variation one can expect in a field planted with an apomict, an embryological analysis (which gives a sexual potential) will not be very useful. However, a progeny test may not be too useful either because in a progeny test plants are usually spaced and competition is very different from that which is realized in a mechanically sown field, with a dense plant population. In *P. maximum,* off-types are always less vigorous than the maternal plants, and due to competition most apomictic biotypes appear obligate when grown in dense swards.

Regardless of whether presence or absence of apomixis is determined by a single dominant gene (Savidan, 1981a, 1981b), the inheritance of the percentage sexual reproduction in apomicts could not be determined because variation within families was greater than variation between families. However, from a practical point of view, and because no important increase in variation could be detected following hybridization, it appeared that most apomictic hybrids could be grown in forage producers fields without concern for variation.

ACKNOWLEDGMENT

The author wishes to express his gratitude to Dr. E. C. Bashaw and Dr. W. W. Hanna for reviewing the manuscript. He also thanks Ernest Anon Amon for technical assistance.

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