



Chromosomal and Embryological Analyses in Sexual × Apomictic Hybrids of *Panicum maximum* Jacq.

Y. Savidan

ORSTOM Research Center, Abidjan (Ivory Coast)

Summary. Cytological analyses in series of crosses between 7 sexual pistillate and 8 apomictic staminate parents of species *Panicum maximum* (Gramineae) are reported. Although these 15 progenitors were tetraploid ($2n = 32$), 2 dihaploids ($2n = 16$), 45 hexaploids ($2n = 48$) and 5 octoploids ($2n = 64$) were observed among 333 progeny plants. The role of unreduced gametes as the originators of polyploidy is discussed in relation to the so-called 'elements of apomixis'. The 2 dihaploids appeared to be sexual while the hexaploids and octoploids were all apomictic. At the tetraploid level sexual and apomictic hybrids segregated in a ratio close to 1:1. These results were then compared to those already obtained from studies on other tropical grasses and indicate a simple genetic determinism for gametophytic apomixis.

Key words: *Panicum maximum* — Embryology — Apomixis — Sexuality — Polyploidization

Introduction

The tropical forage crop *Panicum maximum* Jacq. (Gramineae) forms an agamic complex of great economic value. It is mostly represented in nature by tetraploid biotypes with $2n = 32$ chromosomes that reproduce by facultative apomixis. Obligate sexuality was first discovered in small natural diploid populations of East Africa by Combes and Pernes (1970) and subsequently at the tetraploid level, in progenies of natural apomicts from South Africa, by Smith (1972) and Hanna et al. (1973).

Crossing sexual tetraploids, obtained from colchicine treatment of the natural diploids, with apomicts has been attempted since 1971 in the Ivory Coast. Preliminary results were discussed in previous reports (Pernes et al. 1975a; Savidan 1975, 1978). The major objectives of this paper were to study the chromosome numbers and the

inheritance of modes of reproduction, apomixis and sexuality, in progenies from series of crosses between seven sexual and eight apomictic tetraploids.

Materials and Methods

More than 500 accessions of *Panicum maximum*, mostly from Kenya and Tanzania, are kept in a field collection in our Research Center (Abidjan, Ivory Coast). Only 22 of them are diploid and entirely sexual. The origins of the different genitors used in our 7 × 8 series of crosses are shown in Table 1.

Cytological methods were preferred to morphological tests for the study of modes of reproduction as they were found to be more convenient and more rapid. The technique using phase contrast microscopy has been described and analysed in previous papers (Herr 1971; Savidan 1975). However, field tests were periodically conducted as controls. Sexual autotetraploids are mostly self-sterile and exceptional self-fertilized products are morphologically well-differentiated from hybrids. As a result we used the following hybridization technique: two inflorescences — one from each parent — were put together in a paper bag and seeds were collected on the female panicles. A minimum of 10 bags were set for each cross combination. Chromosome numbers were observed after fixation in Carnoy (6:3:1) and hematoxyline coloration. The sexual or apomictic behaviour was studied by observing the structure of twenty ovules from each hybrid.

Results

Ten crosses didn't produce any hybrids and conspicuous differences were observed involving both success of hybridization and ploidy levels (Table 2).

1) *Success of hybridization:* under our experimental conditions, IS4 was the most productive sexual genitor and yielded ten times more hybrids than the autotetraploid T34.T. This, along with the 10 crosses that didn't produce any hybrids could have resulted from differences in respective seed productivities of pistillate genitors. The diploid K189, from which K189.T, S2.T, P2, IS3 and IS4

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O. R. S. T. O. M. Fonds Documentaire

23 MARS 1982
N° : 82/50/01103

Cote : 13

O. R. S. T. O. M. DEC. 1980

0040-5752/80/0057/0153/\$ 1.00
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originated, was known to yield higher amounts of seeds than any other diploid of our collection. The numbers of hybrids obtained from each apomictic tetraploid was not reported here since no apparent differences were shown.

2) *Ploidy levels*: fifty-two of the 333 plants produced had a ploidy level different from that of the parents, i.e. $2n = 4x = 32$.

Two dihaploids ($2n = 16$) were vigorous and fully fertile. They looked morphologically very similar to our natural diploids. As no characteristic from the staminate parent could be detected, one can assume they arose from parthenogenesis. It must be noted that 60 dihaploids were previously observed in *Panicum maximum* (Pernes et al. 1975b). They arose from a presumed natural interspecific hybrid between *P. maximum* and *P. infestum*. In spite of a regular meiosis, with eight bivalents, all of them were fully sterile. Further embryo sac analyses showed they were potentially apomictic.

Forty-five hexaploid hybrids ($2n = 48$) were observed. They probably resulted from fertilization of unreduced

Table 1. Origins of the fifteen genitors. Accessions preceded by letters K and T were brought back from two trips to Kenya and Tanzania in 1967 and 1969. The apomictic group used as staminate parents is highly polymorphic

Sexual genitors	
T34.T	Vugiri, Tanzania, diploid treated with colchicine
T44.T	Korogwe, Tanzania, diploid treated with colchicine
K189.T	same origin
S2.T	seed from self fertilization of diploid K189, treated with colchicine
P2	sexual hybrid from a K189.T × G23 cross, G23 being an introduction from Kenya
IS3	sexual hybrid from a K189.T × G3 cross, G3 being an introduction from Nigeria
IS4	Full sib of IS3
Apomictic genitors	
K211	south of Mombasa, Kenya
G17	introduction from Kenya
K4	Nairobi, Kenya, a presumed natural interspecific hybrid with species <i>Panicum trichocladum</i>
C1	spontaneous tetraploid appeared in a collection of sterile dihaploids from presumed natural interspecific hybrids with species <i>Panicum infestum</i>
280	introduction from Kenya
T25	Bagamoyo, Tanzania
K26	Between Rumuruti and Maralal, Kenya
K20	Between Rumuruti and Thomson's falls, Kenya

egg cells. They grew vigorously and some of them were selected for agronomic interest. The 5 octoploids ($2n = 64$) were obtained from the same staminate parent, i.e. C1, which is presumed to be a natural interspecific hybrid.

Embryological studies indicated that dihaploids were fully sexual while hexaploids and octoploids were all apomictic. In tetraploid hybrids, including aneuploids with 30, 31 or 33 chromosomes, reproduction was either sexual or apomictic. Table 3 shows the segregations observed in the tetraploid progenies. Isogenic artificial sexual tetraploids are grouped on one side and the three sexual hybrids on the other. A segregation close to 1:1 is observed in the F1 hybrids as well as in the three-way hybrids. It should be pointed out that among the 281 tetraploid hybrids analysed, 141 were sexual and 140 apomictic.

Discussion

Our experiments produced new information at three levels: (1) the level of unreduction in sexual plants, as related to polyploidization, (2) the relation between unreduction and embryo sac structure, and (3) the inheritance of apomixis, in *Panicum maximum*.

Unreduction and Polyploidization

In a review on the origin of polyploidy, Harlan and De Wet (1975) mentioned 68 genus in which hybrids obtain-

Table 2. Numbers of hybrids from each sexual genitor and their ploidy level. The difference between rates of chromosomal abnormalities in progenies from (1) isogenic sexual tetraploids, and (2) sexual hybrids, is highly significant ($\chi^2 = 38.95$)

$2n =$	16	32	Aneu	48	64	Sum
T34.T	0	5	1	3	0	9
T44.T	0	2	1	13	1	17
K189.T	0	25	6	2	2	35
S2.T	1	32	4	14	0	51
P2	0	35	3	4	2	44
IS3	1	70	4	8	0	83
IS4	0	86	7	1	0	94
Sum	2	255	26	45	5	333
		32	≠	Sum		
(1)	64	48		112		
(2)	191	30		221		
Sum	255	78		333		

Table 3. Numbers of tetraploid hybrids (including aneuploids) for each class of sexual genitors, with their apomictic/sexual ratio, tested for a 1 to 1 segregation

Female parents	n	APO	SEX	χ^2 (1:1)
Isogenic sexual tetraploids	76	45	31	2.58 NS
Sexual hybrids	205	95	110	1.10 NS

ed from the fertilization of unreduced egg cells were obtained. These authors thus claimed that polyploidy did not especially result from chromosome doubling after hybridization, as stated by Stebbins (1950), but from fertilization of unreduced gametes. The production of such gametes were seemingly strongly correlated with remote hybridization. Any sexual plant could produce abnormal gametes since meiosis is never hundred per cent successful.

In our preliminary experiments (Pernes et al. 1975a; Savidan 1975), we did not see such chromosomal abnormalities, perhaps because the parents of the crosses were not as distantly related as those used in the new series of crosses. The choice of the eight apomictic staminate parents in these crosses can itself explain the occurrence of 50 hybrids with a $2n + n$ or $2n + 2n$ structure. The rate of unreduction (15.0 per cent) is quite similar to that observed in other species of the *Bothriochloa-Dichanthium* complex (Harlan and De Wet 1975).

Unreduction and Embryo sac Structure

As unreduced egg cells can result from apospory or diplospory, hybrids coming from fertilization of unreduced gametes are often looked upon as a sign of a 'tendency towards apomixis'; the unreduction process being regarded as a so-called 'element of apomixis'. (Grober et al. 1974; Petrov et al. 1978). Thus it does not always seem easy to separate apomixis from a 'sexual polyploidization' process, as defined by Mendiburu and Peloquin (1976). Our experiment perhaps brings a new element to the discussion.

Panicum maximum, like other facultative aposporous Panicoideae, is characterized by a gametophytic dimorphism. Embryo-sacs of aposporic origin, which are the more frequent, are 4-nucleate: one egg, 2 synergids and a unique polar nucleus. Embryo-sacs of sexual origin are 8-nucleate. They have antipodal cells and 2 polar nuclei (Warmke 1954). Numerous cytological observations were made on the T44.T genitor, as it had a high rate of polyploid hybrids production (14 among 17 plants, see Table 2). Several hundred ovules showing only 8-nucleate embryo-sacs were observed. Our suggestion is that unre-

duction in the sexual polyploidization process and unreduction in the apomictic process could be differentiated as they are related to two morphologically different types of embryo-sacs in such material. In the former case, following meiosis, unreduced cells are produced that develop into a 8-nucleate structure; in the latter case, all megaspores abort and are replaced by a nucellar cell that develops into the typical 4-nucleate structure.

We are now convinced that two different types of unreduction exists in plants, one of which is purely sexual. That is why we assert that looking for apomixis in hybrid progenies that are partly composed of $2n + n$ products is absolutely illusive. Apomixis, as defined by Gustafsson (1946) and accepted by almost all specialists, is the result of both unreduction and unfertilization. Therefore one has to be careful in using the term 'element of apomixis'. This term should be abandoned.

Inheritance of Apomixis

Studies conducted since the 50's on such Gramineae as *Paspalum notatum* (Burton and Forbes 1960), the *Bothriochloa-Dichanthium* complex (Harlan et al. 1964), *Cenchrus* (= *Pennisetum*) *ciliaris* (Taliaferro and Bashaw 1966; Read and Bashaw 1969) and *Eragrostis curvula* (Voigt and Bashaw 1972) suggest that one should reconsider the agronomic interest of gametophytic apomixis, at least for aposporous Panicoideae (Savidan 1978). Almost all forage and grain crops of intertropical areas belong to this subfamily. Two improvement programmes have already been successful in these plants, one in the genus *Cenchrus* (Bashaw 1975); the other in *Panicum maximum* (Pernes et al. 1975c; Savidan 1978). Two other examples of a simple genetic determinism were recently given, one in the diplosporic *Tripsacum dactyloides* (Gramineae) by Petrov et al. (1978), the other by Nogler (1978) in a dicotyledon: *Ranunculus auricomus*.

The determinism of apospory in *Panicum maximum* appeared to be simple, although not yet completely analyzed. Anyhow, there could be more than a simple allelic dosage for the expression of apomixis. Since no meiotic abnormalities have been shown in the staminate parents, the hexaploids certainly received twice as much information from the sexual pistillate parent than from the apomictic staminate parent. In spite of such a convenient allelic dosage, sexuality can not be expressed in these hexaploids.

Our crosses especially demonstrated that apomixis and sexuality could be easily managed in an improvement programme, as two and three-way hybrids of *Panicum maximum* were produced. These hybrids had a low rate of sexuality when apomictic and good grass productivity (Savidan 1980), and therefore could be of special commercial interest.

Acknowledgement

I thank Mr. E. Anon Amon for his technical assistance, Prof. J. Pernès and Dr. A. Charrier for their helpful comments and discussions.

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Received October 20, 1979

Accepted January 28, 1980

Communicated by Å. Gustafsson

Dr. Y. Savidan
ORSTOM Research Center,
B.P. V51
Abidjan (Ivory Coast)