ANALYSIS OF REPRODUCTIVE ISOLATION BETWEEN PEARL MILLET (*PENNISETUM GLAUCUM* L.) AND *P. RAMOSUM*, *P. SCHWEINFURTHII*, *P. SQUAMULATUM*, *CENCHRUS CILIARIS*.

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INTRODUCTION

A previous survey (1) of the compatibility between pearl millet pistils and pollens from some other *Poaceae* has shown a high compatibility of pollens from *Pennisetum ramosum, P. schweinfurthii and P. squamulatum* with a diverse array of pearl millet accessions. Pollen from a *Cenchrus ciliaris* accession was found to be compatible only with specific pearl millet accessions. Compatible interspecific crosses between pearl millet and these four species have been observed to evaluate the frequency of ovules fertilized, the final development of embryos and endosperms, the possibility of embryo rescue and the fertility of the interspecific hybrids eventually obtained.

MATERIALS AND METHODS

The three Pennisetum used as males have been crossed to diploid and tetraploid pearl millets. *Cenchrus ciliaris* has been crossed only to a compatible diploid pearl millet from Togo previously identified (1). No tetraploid copy of that accession was available and the other tetraploid pearl millets available were incompatible with *C. ciliaris* pollens (Table 1).

Table 1. Origin of the material used as parents of interspecific crosses with pearl millet.

Material	**Species**	**Source of plant material**
D1	*Pennisetum glaucum* L.	Orstom diploid male sterile line on V cytoplasm
D'1	*Pennisetum glaucum* L.	Maintainer diploid line of D1
D2	*Pennisetum glaucum* L.	diploid accession (Orstom PE 3301) from Togo
T1	*Pennisetum glaucum* L.	tetraploid Indian line from Icrisat (IP 12433)
T2	*Pennisetum glaucum* L.	tetraploid Indian line from Icrisat (IP 12434)
PS 63	*Pennisetum ramosum* (Hochst.) Schweinf.	Tifton, USA
PS 62	*Pennisetum schweinfurthii* Pilger	Tifton, USA
PS243	*Pennisetum schweinfurthii* Pilger	Tifton, USA
PS244	*Pennisetum schweinfurthii* Pilger	Tifton, USA
PS 24	*Pennisetum squamulatum* Fresen.	Tifton, USA
M49	*Cenchrus ciliaris* L.	accession from Agadès (Niger), collected by Orstom

About 10 days after pollination, the ovules have been dissected in sterile conditions and observed with a binocular lens for the presence and dimensions of embryos and endosperms. A few ovules were also observed by cytological fixation-inclusion methods. The embryos found were excised and grown in vitro on embryo rescue medium (2). The chromosome doubling of a sterile hybrid has been made by somatic embryogenesis from immature inflorescences after colchicine treatment during callogenesis (1 g/l for 5 days).

RESULTS

P. ramosum crossed with a tetraploid millet has given many big embryos without endosperm.The embryo rescue of 48 embryos produced 31 F1 plants male sterile but partially female fertile when pollinated by diploid millet -about 39 seeds on each pollinated spike- (Table 2).

P. schweinfurthii crossed with a diploid millet gave many mature almost normal seeds: 80 seeds sown 3 months after harvest gave 12 plants. The embryo rescue of 29 embryos excised 30 days after pollination gave 19 hybrid plants. All these F1 plants were totally sterile. One amphidiploid plant could be obtained by chromosome doubling technique. That amphidiploid plant was completely male sterile but gave many seeds when pollinated by a tetraploid millet or by a hybrid between a tetraploid millet and *P. squamulatum* but very few seeds when pollinated by a diploid millet. One hybrid could be obtained by embryo rescue in a cross between a tetraploid millet and *P. schweinfurthii* but it was completely sterile.

P. squamulatum crossed with a diploid millet gave a high frequency of small embryos with large undifferentiated endosperms and no plant could be rescued. Crosses with tetraploid millets gave plenty of normal seeds. Those F1 hybrids were partially fertile. They gave many seeds when pollinated by sib-mating or by tetraploid millets but rare seeds with a diploid millet pollinator.

Cenchrus ciliaris crossed with the compatible diploid millet (D2 line) gave a high frequency of aborted seeds similar to those of the cross diploid millet x *P. squamulatum*. It is suspected that a cross D2 tetraploid *x C. ciliaris* would give plenty of normal seeds.

Table 2. Rate of fertilized ovules (%) and length of embryos N days after pollination on the crosses observed. N= number, Min=minimum, Max=maximum. Standard deviation in parentheses.

Material	**N Days**	**N Ovaries**	**N embryos**	**%**	**Embryo**	**length**	**(mm)**
					Min	**Mean**	**Max**
Controls							
D1xD1	19		20		1.5	1.95 (0.21)	2.3
T1 x T1	30		20		1.9	2.28 (0.21)	2.6
Hybrids	with	*P. ramosum*					
D1-1 x PS63	8	168	14	8		0.35	
T1-3 x PS63	13	50	8	16	0.5	0.62 (0.13)	0.8
T1-7 x PS63	12	59	35	59	0.3	1.07 (0.36)	1.6
T2-1 x PS63	14	171	30	18	0.41	0.50 (0.11)	0.75
Hybrids	with	*P. schweinfurthii*					
D1-8 x PS62	17	123	32	26	0.2	0.86 (0.41)	1.60
D1-10 x PS243	17	80	36	45	0.4	1.15 (0.27)	1.60
D1-2 x PS244	8	88	31	35	0.7	1.0 (0.19)	1.35
T1-5 x PS62	16	220	60	27	0.4	0.92 (0.28)	1.35
T1-7 x PS243	11	219	51	23	0.2	0.51 (0.30)	1.2
T1-2 x PS244	14	95	24	25	0.2	0.56 (0.23)	1.0
Hybrids	with	*P. squamulatum*					
D1-3 x PS24	8	81	59	73	0.44	0.46 (0.02)	0.49
T1-5 x PS24	30	seed set and grain size similar to controls					
Hybrids	with	*Cenchrus*	*ciliaris*				
D2-9 x M49	7	21	7	33			
D2-2 x M49	9	15	11	60		0.2	

A RAPD analysis with the 9 primers A19, K3, K6, J13, M12, M13, X9, Y15, Z6 gave 76 bands characteristic of the interspecific hybrids: 31 Ramosum bands, 19 Squamulatum bands and 26 Schweinfurthii bands (Table 3).

Table 3.

	A1 9	K3	K6	J13	M12	M13	X9	Y15	Z6	T	A19	K3	K6	J13	M12	M13	X9	Y15	Z6	T	A19	K3	K6	J13	M12	M13	X9	Y15	Z
					GxR										GxSq										GxSchw				
N bands	7	5	7	5	12	2	7	10	5	60	4	5	5	8	5	3	1	9	2	42	5	5	1	6	5	5	6	7	5
Glauc	3	3	2	2	6	1	3	7	2	29	2	2	3	3	4	1	1	6	1	23	2	1	1	2	4	2	1	3	3
Ram	**4**	**2**	**5**	**3**	**6**	**1**	**4**	**3**	**3**	**31**	-	-	-	-	-	-	-	-	-										
Squam	-	-	-	-	-	-	-	-	-		**2**	**3**	**2**	**5**	**1**	**2**	**0**	**3**	**1**	**19**									
Schw																					**3**	**4**	**0**	**4**	**1**	**3**	**5**	**4**	**2**

DISCUSSION

The first isolating mechanism acting between pearl millet and the four species here considered was postzygotic abortion due to endosperm defects which could be reduced by an adequate ploidy level of parents or by embryo rescue. The second mechanism, encountered in pearl millet x *P. schweinfurthii* hybrids, was hybrid sterility due to absence of chromosome homology which could be treated by chromosome doubling. It is suspected that the male sterility of the amphiploid plant obtained could be due to the V male sterile cytoplasm of the pearl millet female parent. It appears that changes in ploidy level of the parents have not modified the level of pistil-pollen compatibility and that the expression of postzygotic abortion varies much between seeds of a same spike.

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