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Allelic relationship of four male sterility genes and nucleo-cytoplasmic interactions in the expression of male sterility in pearl millet, *Pennisetum americanum* (L.) Leeke

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Summary. Male sterility genes isolated in four inbred lines of pearl millet were found allelic. The differences between male fertile and male sterile phenotypes is mainly due to a single gene. Presence of a dominant gene (*Ms*) resulted in male fertility and double recessiveness (*ms ms*) in male sterility. However, genic male sterility (GMS) in *Pennisetum* is not a simply inherited case of monogenic recessive condition but is influenced by cytoplasmic and several nuclear factors. In a male sterile, the stage at which the male sterility gene is expressed during the development of the male gametophyte resulting in breakdown of the cells is influenced by cytoplasmic and other nuclear factors. Two types of cytoplasm, C-1 and C-2, are recognized. Presence of any two recessive male sterility alleles in C-1 led to breakdown of male development before differentiation of an archesporium in the anther (Arc-type); in C-2 cytoplasm, degeneration started during meiosis with fusion of meiocytes and syncyte formation (Syn-type), or at post-meiotic stages terminating in abortion of microspores before first pollen mitosis (PGM type). The triggering of activity of recessive male sterility genes in C-2 cytoplasm appeared to be regulated by two nuclear factors, R_1 and R_2 with duplicate gene action. Recessiveness for both the R factors in C-2 cytoplasm resulted in PGM-type expression. The action of R_1 and R_2 is specific to C-2 cytoplasm. Mutation of cytoplasm from C-1 to C-2 and C-2 to C-1 was observed.

Key words: Pearl millet – Genic male sterility – Allelism – Cytoplasmic factors – Cytoplasmic mutation

Introduction

Gottschalk and Kaul (1974) in their review on genic male sterility (GMS) observed, that while many male sterility genes have been isolated in crop plants, allelism tests have been done in very few cases. Tsuchiya (1981) noted that in many cases researchers paid little or no attention to allelic relationships and genetic backgrounds of a particular mutant or interaction of genes in character expression; he emphasized the importance of allelism testing, a simple and basic practice in genetic studies of plants. Recognition of the potential of GMS in plant breeding of late has led to several studies on allelic relationships of male sterility genes. Since male sterility results from breakdown of male development at various stages, specific to each gene, study of cytological expression and allelic relationships of different male sterility genes aids in understanding of genetic control of microsporo- and gametogenesis (Golubovskaya and Mashnenkov 1981; Golubovskaya and Urbach 1981; West and Albertsen 1985).

Allelic relationships of four male sterility genes of independent origin in pearl millet, an important grain and fodder crop of the tropics, and observations relating to the effect of cytoplasmic and nuclear factors on the expression of male sterility are reported in the present paper.

Materials and methods

The allelic relationship of the male sterility genes isolated in the four inbred lines, Vg 272, IP 482, PDP and IP 457, was studied. Male sterility in all lines except PDP originated spontaneously. In PDP it was induced by sodium azide (Subba Rao 1985). Male sterility in all lines was reported to be due to a recessive gene (Krishna Rao and Koduru 1978a, b; Uma Devi 1981; Subba Rao 1985), but frequent distortions from monogenic segregation occurred due to influence of environmental and modifying gene factors (Uma Devi 1981; Krishna Rao and Uma Devi 1983).

Male sterility in the line Vg 272 was described as PGM-type (Krishna Rao and Koduru 1978 a). The same line, re-examined

several years later for cytological details, showed Arc-type expression (Krishna Rao and Uma Devi 1983). Since this was an unexpected change in the expression of male sterility, cytological details were examined routinely in all male sterile plants in subsequent generations. Male sterility in this line continued to be expressed as Arc-type for seven generations, but in the eighth generation in one of the segregating families one plant showed reversion to PGM-type expression, similar to the original type (Krishna Rao and Uma Devi 1983).

Male sterility in IP 482 was Syn-type while that in PDP was of PGM-type resembling the one reported in Vg 272 (Subba Rao 1985). In IP 457, Arc-type male sterility identical to that in Vg 272 was observed.

Allelism tests were carried out by crossing male sterile of one inbred line to a heterozygous male fertile of another inbred line. Reciprocal crosses were also made in each case. A 1:1 segregation of male fertiles and steriles in F_1 indicates that the genes under consideration are allelic. Replicates of some of the progenies that showed deviations from the expected segregations were raised in different seasons.

The effect of cytoplasm on the expression of male sterility gene was tested by introducing the recessive male sterility gene in the line IP 482 (Syn-type) into the cytoplasmic background of the inbred line SAR 1134. This was accomplished by crossing SAR 1134 as female to heterozygous male fertile IP 482. The expression of male sterility gene was studied in the F_2 of this cross. Only half of the F_1s are expected to carry the recessive male sterility allele of IP 482. Therefore, F_2 families were raised from five F_1s.

Cytological details were examined in aceto-carmine squashes of anthers after the ears were fixed in acetic-alcohol for at least 24 h.

Results

Allelic relationships

The F_1s of the crosses made to test the allelic relationships of the male sterility genes in Vg 272 (Arc-type) and IP 482 (Syn-type) showed segregation for male sterility. Segregation approximated the ratio of 1 fertile:1 male sterile, showing that the male sterility genes in the two lines are allelic (Table 1).

The male steriles segregating in the reciprocal crosses are genotypically similar with respect to the male sterility genes. However, they differed in their cytological expression and resembled the female parent in each case. The male steriles segregating in the F_1 when Vg 272 was the female parent were Arc-type; when IP 482 was the female parent, they were mostly of Syn-type but a few showed PGM-type expression.

The causes for the difference in expression of male sterility in the reciprocal hybrids and for the unexpected appearance of PGM-type expression were investigated in the F_2, F_3 and sib-mated families. The F_1 male steriles were also crossed to heterozygous male fertiles of the parental lines. The segregation pattern observed in several of the families further supports the allelism of male sterility genes in the two lines (Table 1). There was a deficiency in the frequency of male steriles in some families. This could be due to failure of expression of male sterility in genotypically male sterile plants because of environmental and modifying gene effects, the role of which had earlier been demonstrated (Krishna Rao and Uma Devi 1983). In all families showing segregation for male sterility, the male steriles mostly resembled the female parent (Table 1), suggesting the influence of cytoplasmic factors on expression of male sterility.

The PGM-type male sterility originally located in Vg 272 (Krishna Rao and Koduru 1978a) and subsequently lost (Krishna Rao and Uma Devi 1983) reappeared in several progenies involving IP 482 as the female parent, though a male sterile of such a type was not involved in the crosses. This kind of male sterility occurred only in the cytoplasmic background of IP 482 but not in Vg 272 in these crosses.

The male sterility genes in (a) PGM-type male sterile of Vg 272, that reappeared in the eighth backcross generation of Vg 272 segregating for Arc-type male sterility (Krishna Rao and Uma Devi 1983), (b) PGM-type male sterile of PDP and (c) Arc-type male sterile of IP 457 proved allelic to the genes governing Arc-type male sterility in Vg 272. Even in these crosses the segregating male steriles resembled the female parent (Table 1). A similar situation of allelic genes governing Arc-type and PGM-type male sterility was reported in *Cucurbita maxima* (Francis and Bemis 1970).

Cytoplasmic effects

Since the results indicated influence of the maternal parent (cytoplasm) on the cytological expression of the recessive male sterility alleles, the gene for Syn-type male sterility of IP 482 was introduced into the cytoplasmic background of another inbred line, SAR 1134, to test the effect of cytoplasm. Of the five F_2 families (from different F_1s) raised of the cross SAR 1134 (♀) × IP 482 heterozygous for Syn male sterility, one showed segregation for male sterility (160 male fertiles: 52 male steriles). All the male steriles were of Arc-type. Thus, the male sterility gene which expressed as Syn-type in IP 482 expressed as Arc-type when transferred into the cytoplasmic background of SAR 1134. Clearly, the stage of action of the recessive male sterility allele is influenced by cytoplasmic factors.

Discussion

The results indicate that the male sterility genes isolated in the various lines of *Pennisetum* are of the same locus. The stage of male development at which the recessive male sterility alleles are expressed seemed to be influenced by cytoplasmic factors. Two types of cytoplasms have been identified. Irrespective of the type of recessive male sterility alleles present in a male sterile, one type of cytoplasm allowed Arc-type expression and the other

Table 1. Results of the allelism test between male sterility genes of the inbreds Vg 272 (Arc- and PGM-types) IP 482 (Syn-type), IP 457 (Arc-type) and PDP (PGM type) in pearl millet. x and y are replicates in two seasons. Ratios in brackets indicate ratio between Syn: PGM male steriles

S. No.	Cross		Family	No. of families	No. of plants		Type of ms	Expected ratio of mf : ms	χ^2
	ms	× heterozygous mf			mf	ms			
1	Vg 272 (Arc)	× IP 482	F_1	4	303	277	Arc	1:1	0.58
			F_2	6	241	72	Arc	3:1	0.67
	IP 482 at S. No. 1 selfed			2	130	31	Syn	3:1	2.84
2	F_1 at S. No. 1	× Sib		1	68	73	Arc	1:1	0.18
3	F_1 at S. No. 1	× Vg 272		2	95	85	Arc	1:1	0.56
				3x	164	0	–	1:1	–
				y	132	26	Arc	1:1	*
4	F_1 at S. No. 1	× IP 482	BC	2	107	32	Arc	1:1	*
				2x	114	0	–	1:1	–
				y	59	16	Arc	1:1	*
	IP 482 at S. No. 4 selfed			4	153	7	Syn	3:1	*
5	IP 482	× Vg 272 (Arc)	F_1	1	28	22	19 Syn; 3 PGM (3:1)	1:1	0.72
				1x	92	28	Syn	1:1	*
				y	38	32	29 Syn; 3 PGM	1:1	0.52
			F_2	2 (Sibs 1; 2)	103	24	Syn	3:1	2.52
				7 (Sibs 3, 4, 5, 6, 7, 9, 11)	362	8	Syn	3:1	*
				1 (Sib 8)	59	12	9 Syn; 3 PGM (3:1)	3:1	2.52
				2 (Sibs 10; 12)	155	13	9 Syn; 3 PGM 1 Arc	3:1	*
			F_3	5 (Sibs 1, 5, 8, 10, 12)	325	13	10 Syn; 3 PGM	3:1	*
6a	F_1 at S. No. 5	× Sib 2		1	26	20	Syn	1:1	0.78
b	F_1 at S. No. 5	× Sibs 8; 10		3	110	102	96 Syn; 6 PGM (15:1)	1:1	0.30
c	F_1 at S. No. 5	× Sib 4		1	43	10	Syn	1:1	*
7	F_1 at S. No. 5	× Vg 272 (Arc)	BC	1	32	27	15 Syn; 12 PGM (1:1)	1:1	0.42
				2	98	0	–	1:1	–
				1x	78	0	–	1:1	–
				y	42	12	10 Syn; 2 PGM	1:1	*
			Selfed BC	3	234	7	6 Syn; 1 PGM	3:1	*
				1[a]	177	54	50 Syn; 4 PGM (15:1)	3:1	0.33
8	PGM ms segregating at S. No. 7	× Vg 272 (Arc)		1	48	55	PGM	1:1	0.48
9	Vg 272 (PGM)	× Vg 272 (Arc)	F_1	2	63	64	PGM	1:1	0.008
			F_2	2	169	56	PGM	3:1	0.14
				1	81	23	Arc	3:1	0.47
10	Vg 272 (Arc)	× Vg 272 (PGM)	F_1	2	67	71	Arc	1:1	0.12
				2x; y	177	44	Arc	1:1	*
11	PDP (PGM)	× Vg 272 (Arc)	F_1	1x	81	0	–	1:1	–
				y	46	7	PGM	1:1	*
			F_2	5	160	49	PGM	3:1	0.27
12	IP 457 (Arc)	× Vg 272 (Arc)	F_1	2	87	108	Arc	1:1	2.26
	Vg 272 at S. Nos. 3, 5, 7 9, 11, 12 selfed			13	433	108	Arc	3:1	9.30**
13	Vg 272 (Arc)	× PDP (PGM)	F_1	2	75	62	Arc	1:1	1.24
	PDP at S. No. 13 selfed			2	44	16	PGM	3:1	0.09

mf = male fertile
ms = male sterile
* = χ^2 with significant P value at 5% level
** = homogeneity χ^2
[a] = Selfed progeny of a BC family showing no segregation for male sterility

type permitted Syn- or PGM-type phenotype. The former is described as C-1 cytoplasm and the latter, C-2. Following this, cytoplasm of the lines Vg 272, IP 457 and SAR 1134 is of C-1 type and that in IP 482, C-2.

The sudden appearance of all Arc-type male steriles in the line Vg 272, expected to show PGM-type and reappearance of PGM-type in one of the segregating male sterile plants in a subsequent generation (Krishna Rao and Uma Devi 1983), can now be explained as follows: The cytoplasm of the line Vg 272 was originally of C-2 type permitting PGM-type expression (Krishna Rao and Koduru 1978 a), but later mutated to C-1 type in the male sterile used for maintaining the mutant. This remained undetected because, subsequent to initial cytological studies, male steriles were recognized and maintained by observing only external anther morphology and seed set on bagged ears (P.R.K. Koduru, personal communication). The line, when cytologically checked after several generations, was found to show Arc-type male sterility. The reoccurrence of PGM-type expression in one of the plants of a later generation can be assumed to be due to a cytoplasmic mutation, from C-1 to C-2. The occurrence of Arc-type male steriles in the F_2 families of the crosses IP 482 Syn-type male sterile (C-2) × Vg 272 heterozygous male fertile (S. No. 5, Sib 10, Table 1) and Vg 272 PGM-type male sterile (C-2) × Vg 272 (S. No. 9, Table 1) can also be explained as due to a cytoplasmic mutation from C-2 to C-1.

Frequent cytoplasmic mutations have been reported in CMS lines of maize (Singh and Laughman 1972) and pearl millet (Clement 1975; Burton 1977). While in these cases cytoplasmic mutation resulted in conversion of male sterile phenotype to fertile, in the present case a cytoplasmic mutation changed the expression of male sterility from one type to another.

GMS in pearl millet is, thus, influenced by cytoplasmic factors. That GMS is the result of nucleo-cytoplasmic interactions was elucidated much earlier by Hermsen (1965). A cytoplasm capable of restoring fertility to GMS barley was found by Pfeifer (1972). That GMS and CMS are essentially similar is clear from the results of Burnham et al. (1981), who explained the inheritance of male sterility in flax as equally well-based on either of the hereditary patterns. The gene-cytoplasmic relation reported now differs from all those cases, in that it does not relate to fertility-sterility status but to different expressions of sterility. Here polymorphic sterile cytoplasms as in CMS lines of maize (C, T, S), *Pennisetum* (A_1, A_2, A_3) and *Origanum vulgare* have been found (Burton and Athwal 1967; Duvick and Noble 1977; Kheyr-Pour 1980).

The occurrence of both Syn- and PGM-type male steriles in IP 482 with C-2 cytoplasm needs explanation. In the families where the two types of male steriles occur, (considering only those families without a deficiency in the expected segregation of male steriles), the frequencies of Syn- and PGM-types fitted genetic ratios of 15:1, 3:1 or 1:1 (Table 1). This suggests the role of duplicate factors in the expression of male sterility. The factors can be symbolized R_1 and R_2. A dominant gene at either or both of these loci allows Syn-type expression of male sterility while recessiveness for both results in PGM-type. It appears that the action of *R* genes is specific to *ms* alleles in C-2 cytoplasm, with no effect either on *Ms* or on *ms* in C-1 cytoplasm. Instances of gene action specific to one cytoplasm are known in literature; for example, in CMS lines the action of maintainer and restorer genes is specific for one type of sterile cytoplasm with no effect on another sterile cytoplasm of the same species. The phenotypes expected of various cytoplasmic and nuclear factor combinations based on this discussion are shown in Table 2.

Table 2. Phenotypes of different gene cytoplasm combinations in pearl millet

Cytoplasm type	Genes at		Phenotype
	Ms locus	*R* loci	
C-1 or C-2	*Ms*−	R_1-R_2-; $R_1-r_2r_2$; $r_1r_1\ R_2-$; $r_1r_1\ r_2r_2$	Male fertile
C-1	*msms*	R_1-R_2-; $R_1-r_2r_2$; $r_1r_1\ R_2-$; $r_1r_1\ r_2r_2$	Arc-type male sterile
C-2	*msms*	R_1-R_2-; $R_1-r_2r_2$ $r_1r_1\ R_2-$	Syn-type male sterile
C-2	*msms*	$r_1r_1\ r_2r_2$	PGM-type male sterile

However, in many families male steriles were not recovered in the expected proportions and in some families were absent altogether. This precluded a detailed study of the *R* factors.

The effect of cytoplasmic and *R* factors was not observed in the earlier study of Krishna Rao and Koduru (1978 a). The gene for PGM-type male sterility of Vg 272 when transferred to the cytoplasm of IP 482 showed the same PGM-type expression. This was because Vg 272 originally carried C-2 cytoplasm and presumably also recessive *r* factors.

The present observations indicate that the mode of expression of genic male sterility in pearl millet is influenced by cytoplasmic factors and possibly by other nuclear factors.

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