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Induction of cytoplasmic male-sterility into ryegrass (*Lolium perenne*)

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Summary. Data are presented which indicate that a cytoplasmic/genetic type male sterility has been induced into backcross progeny derived from intergeneric hybridization between *Festuca pratensis* (female parent) and *Lolium perenne*. Large numbers of male sterile genotypes have been obtained in all the backcross generations examined. The frequency and purity of maintainer genotypes is low and requires further breeding and selection. Analysis of data suggest that at least two loci are involved in fertility restoration. Conclusions regarding the genetic model are tentative and require further analyses.

Key words: Male sterility – *Lolium perenne* – Maintainers – Restorers – Intergeneric hybridization

Introduction

The number of species in which cytoplasmic-genetic male sterility (CMS) has been reported is quite large (Edwardson 1970). This reflects the continued interest in techniques which facilitate the production of hybrid seed in commercial quantities. Nitzsche (1971) first reported CMS in *Lolium multiflorum*. Wit (1974); Gaue (1977, 1981) reported the occurrence of CMS genotypes in *L. perenne* and discussed their use in hybrid breeding in this species. In all these reports the cytoplasm of the male sterile stocks was derived from the *Lolium* species. This paper reports on the results of experiments designed to induce CMS into *L. perenne* following intergeneric hybridization.

Materials and methods

There are many examples in the literature in which CMS has been found as a result of interspecific/intergeneric hybridization followed by backcrossing to the species used as pollen parent (Lacadena 1968). In 1969, pair crosses between *F. pratensis* (as maternal parent) and *L. perenne* were made following emasculation. Six F_1 seedlings were established from the resulting seed. These F_1 hybrids differed in morphology and pollen fertility as measured by anther development and staining (in safranin) of the contents of the anthers. Two plants produced no anthers but were also sterile as female parents and repeated attempts over several years to obtain backcross progeny failed. The other four F_1 hybrids were backcrossed to *L. perenne* genotypes taken at random from the plant breeding nursery. On some occasions backcrossing was by open pollination to ryegrass. In each backcross generation progeny families were screened for male sterility using anther development and staining of squashed anthers as a measure of this character.

Classification of male steriles

In the first four backcross generations plants were graded on the basis of anther morphology into three groups as follows: fertile (F), semifertile (SF) and sterile (S). Samples of anthers from male sterile genotypes (shrivelled, non-dehiscent, translucent anthers) were macerated in a drop of safranin and examined microscopically for the presence of normal pollen. There was good agreement between visual classification of anthers and microscopic examination of male steriles.

In the fifth and subsequent backcrosses where large numbers of paircross families were produced, a standard method of classification based on anther characteristics only was used. This system is similar to that used in sugar beet (Bosemark 1972). Based on this scheme plants were graded into four classes as follows.

Class 1: male sterile: Anthers are flat, non-dehiscent, shrunken, usually white or translucent with thin walls. These contain no pollen (Fig. 1 a, b).

Class 2: male sterile: Anthers are non-dehiscent, shrunken but not quite as flat as in class 1. Anthers contain no viable pollen, sometimes empty pollen cases are present on staining (Fig. 1 c, d).

Class 3: semi-male steriles: Anthers are more or less normal in shape, contain some pollen (not necessarily viable) when

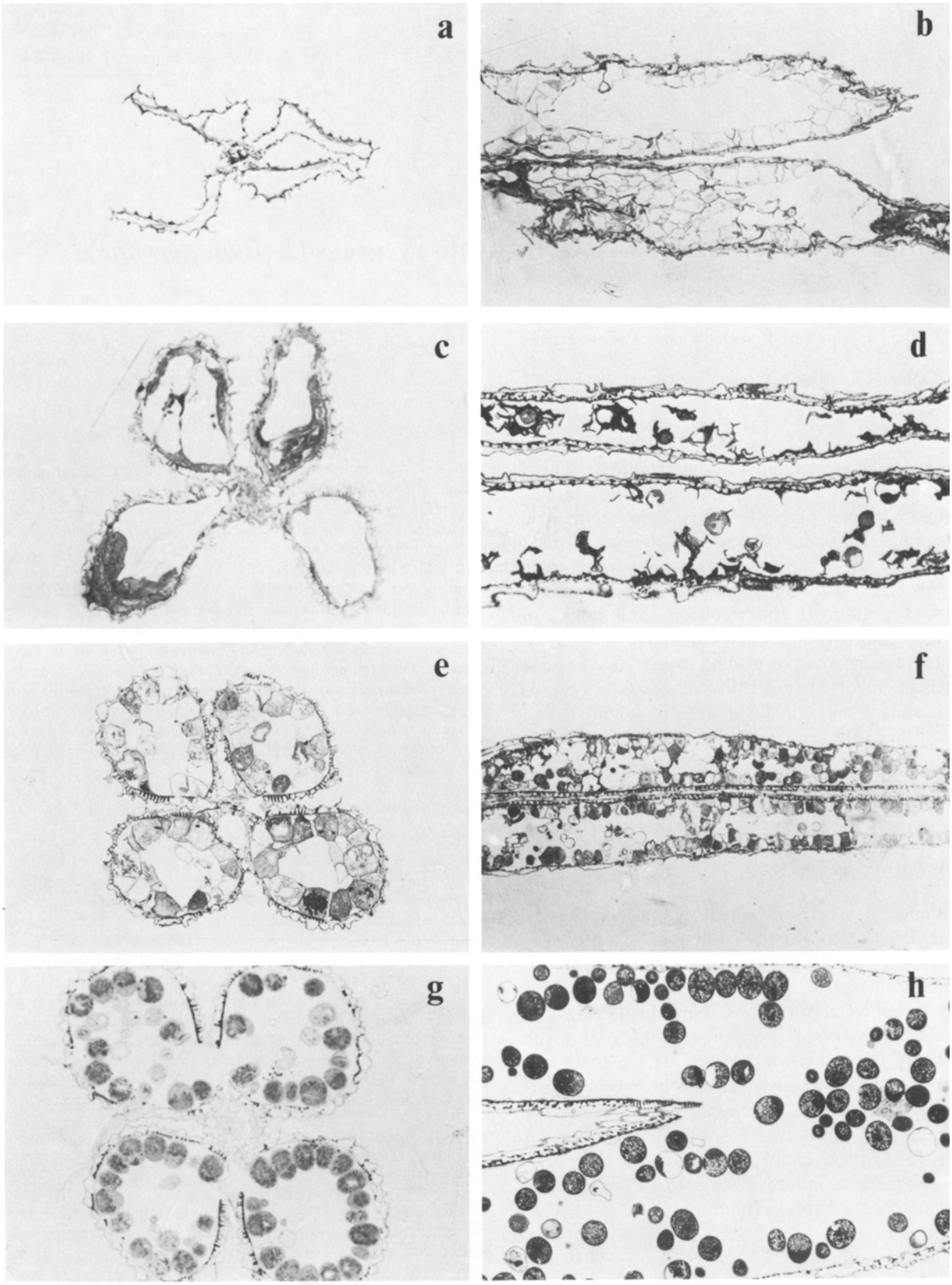

Fig. 1. Transverse and longitudinal sections (×30) of male sterile anthers class 1 **(a, b)** and class 2 **(c, d)**, semi-male sterile anthers class 3 **(e, f)** and fertile anthers class 4 **(g, h)**

stained. Anthers dehisce at one or both ends giving a "spoon" type appearance to the anther (Fig. 1 e, f).

Class 4: Fertile: Anthers normal in shape & colour, well filled and release pollen after dehiscence (Fig. 1 g, h).

Results

In the early backcross generations the number of plants per family was small. Most families showed segregation for male fertile/male sterile genotypes. The frequency of male sterile genotypes in each of the first four backcross generations is summarized in Table 1.

The frequency of sterile genotypes declined from the second backcross onwards. In the fourth generation some families were all fertile, in addition one family was obtained in which all the progeny were male sterile (pollinator A19). Using selected BC_4 male steriles 59 pair cross families each with approximately 21 genotypes were raised in the fifth backcross. The pollen parent A19 was widely used in this backcrossing to determine if its non-restoring character as observed in BC_4 was repeated. The overall results for all families are summarised in Table 2 using the four group classification scheme as defined earlier.

Analysis of 5th generation progenies

The frequency of families in which all plants are sterile (class 1 and 2 type anthers) is low. There are equally few families in which all genotypes are fertile. The family type of greatest frequency shows segregation into all four classes. For analysis of segregation ratios, classes 1 and 2 were combined into one group (sterile) classes 3 plus 4 into another group (fertile). The segregation pattern for each of 23 families is summarized in Table 3. All progenies of A19 were excluded from this table and were analysed separately.

With the exception of two families the segregation pattern agrees with either a 1 : 1 or 3 : 1 theoretical ratio. The 3 : 1 ratio suggests that there are at least two loci involved in fertility restoration. It is assumed also that dominance for fertility restoration is complete and that one dominant allele at either locus results in fertility. This genetic model is probably an over simpli-

Table 1. Frequency of fertile (F), semi-fertile (SF) and sterile (S) genotypes in backcross generations 1 to 4

Generation	F	SF	S	Total	% Sterile
BC_1	13	7	26	46	57
BC_2	21	23	81	125	65
BC_3	241	79	141	461	31
BC_4	381	37	83	501	17

Table 2. Frequency distribution of families of fifth backcross progenies into four classes as defined in text

Anther type	No. of families	No. of progenies in each class				
		1	2	3	4	
Class 1 only	2	33	–	–	–	All Sterile
Class 1 and 2	3	55	3	–	–	
Class 1 and 3	1	19	–	2	–	Segregating
Class 1 and 4	3	35	–	–	22	
Class 2 and 3	0	–	–	–	–	
Class 2 and 4	1	3	–	–	16	
Class 3 only	0	–	–	–	–	
Class 3 and 4	0	–	–	–	–	
Class 1, 2 and 3	3	45	12	6	–	
Class 1, 2 and 4	12	96	35	–	100	
Class 1, 3 and 4	1	10	–	2	6	
Class 2, 3 and 4	1	–	3	2	15	
Class 1, 2, 3 and 4	28	199	80	58	219	
Class 4 only	4	–	–	–	84	All fertile
Totals	59	495	133	70	462	

Table 3. Segregation pattern for 23 families

Family no.	Fertile	Sterile	Agreement with[a]:	
			1:1	3:1
5B2	10	10	NS	**
5B5	8	13	NS	***
5B6	18	3	***	NS
5B8	11	10	NS	*
5B9	15	6	*	NS
5B13	10	11	NS	**
5B14	15	5	*	NS
5B15	9	12	NS	***
5B17	4	17	**	***
5B20	12	9	NS	*
5B26	10	11	NS	**
5B36	8	13	NS	***
5B37	11	10	NS	*
5B42	10	9	NS	*
5B44	11	10	NS	*
5B45	6	15	*	***
5B50	10	2	*	NS
5B51	7	11	NS	***
5B52	7	14	NS	***
5B54	10	6	NS	NS
5B57	18	2	***	NS
5B62	8	13	NS	***
5B64	14	6	NS	NS

[a] Test of observed ratio for agreement with either 1:1 or 3:1 hypothetical segregation

*, **, *** Observed ratio differs significantly at $P<0.5$; 0.1; 0.01, respectively

NS = not significant

fication, however there is reasonable agreement in most families. The two families (5B17 and 5B45) which do not agree with the above segregation pattern have a high frequency of sterile genotypes.

Of the 50 families in Table 2 which show segregation, 16 have ratios significantly different from both a 3 : 1 or 1 : 1 ratio. All but one of these 16 have a very high frequency of sterile genotypes and in 9 of these families genotype A19 was the pollen parent. The data relevant to genotype A19 is given in Table 4.

The results indicate that A19 is a maintainer although not a perfect one. Gaue (1977) also found a low frequency of semi-fertile or fertile genotypes in the progeny of maintainer plants. Similar type results have been recorded in Sugar beet (Bosemark 1972; Owen 1945, 1950). Crossing the male sterile 4B 33/14 to A19 produced all male sterile progeny but similar crosses using S24/2 and M5 as pollinators resulted in fully fertile families indicating that these two plants were restorer genotypes. In addition A19 was crossed to two male fertile plants from a segregating family (4B 34/3 and 4B 34/6). The progenies showed segregation into fertile and sterile types as might be expected on the assumption that A19 was recessive and the female parents heterozygous for the nuclear genes controlling fertility.

Genotype A19 and two of its selfed progeny were used as pollen parents in a second paircrossing programme. This data is summarized in Table 5. Seventy-seven percent of all the plants were sterile (class 1 plus 2) while 23% were class 3. The absence of fertile class 4 genotypes in any of the second generation progeny indicates that this parent (A19) is a maintainer. The occurrence of some class 3 genotypes at a high frequency in a few families would suggest that modifying factors are present and selection for more stable expression of the non-restoring character is necessary.

Table 4. Classification of families derived from A19 as pollen part plus other relevant crosses

Male sterile		Pollinator	Anther classification			
			Sterile		Fertile	
			1	2	3	4
4B 33/5	×	A19	15	1	1	2
4B 33/8	×	A19	17	1	–	–
4B 33/9	×	A19	15	2	3	1
4B 33/10	×	A19	19	1	–	–
4B 33/14	×	A19	17	3	1	–
4B 33/16	×	A19	17	1	–	2
4B 33/17	×	A19	15	–	–	1
4B 33/19	×	A19	19	–	2	–
4B 33/20	×	A19	12	–	–	–
Total			146	9	7	6
4B 33/14	×	A19	17	3	1	–
4B 33/14	×	S24/2	–	–	–	21
4B 33/14	×	M5	–	–	–	21
Male fertile						
4B 34/3	×	A19	2	2	1	13
4B 34/6	×	A19	2	5	2	10

Table 5. Classification of pair cross families derived from A19 or A19 selfed plants used as pollinators

Cross			Classification (anther type)			
Male sterile		Pollinator	1	2	3	4
5B 18-9	×	A19	7	3	14	0
5B 18-16	×	A19	5	16	2	0
5B 40-5	×	A19	7	9	4	0
5B 40-20	×	A19	10	5	2	0
5B 40-21	×	A19	5	14	5	0
5B 40-5	×	A19⊕4	10	10	1	0
5B 60-11	×	A19⊕4	9	10	5	0
5B 62-11	×	A19⊕4	5	13	6	0
5B 63-8	×	A19⊕4	1	21	2	0
5B 62-1	×	A19⊕12	3	10	11	0

Discussion

The results presented indicate that male sterility of a cytoplasmic/genetic nature has been induced following the intergeneric hybridization *F. pratensis* and *L. perenne*. Analysis of segregating families would suggest that there are at least two loci involved in fertility restoration. This does not explain all the results obtained and this genetic model must be regarded as tentative. It is probable that other loci with minor effects have a modifying influence on the expression of the character and it will be necessary to develop inbred or partially inbred lines in order to have more precise genetic analyses. The frequency of maintainers is low. In this series of pair crosses to non-inbred randomly chosen pollen parents only three genotypes which appear to be non-restorers were found, one of which (A19) was analysed in some detail. More recent work involving more than 1,000 paircross families indicates that the frequency of maintainers is probably in the region of 2%. This contrasts with the results of Wit (1974) in which he found the frequency of B types (type 0) to be rather high in existing ryegrass varieties. All of the evaluation and screening of progenies was done in the greenhouse under conditions which in general would favour anther dehiscence (Gaue 1977). It is probable that the classification scheme used may

tend to over estimate the fertility levels present in some cases. Several pair crosses between class 3 genotypes were made but only one showed seed set which suggests that many of the class 3 types may be effectively sterile.

From the applied aspect it is essential that good quality male steriles and appropriate maintainer lines be established in order to allow large scale production of male sterile seed stocks. The objective is to obtain combinations which give 100% class 1 type male steriles. Our results indicate that the maintainers found are somewhat less than perfect and further selection within progenies derived from interbreeding and/or inbreeding potential maintainers will be necessary in order to extract more refined stable non-restorers.

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