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MALE STERILITY IN PEA VIII: DETERMINISTIC DEGENERATIVE MALE MEIOSIS

M.L.H. KAUL, C.NIRMALA and VARINDER MOHAN
Botany Department,University Kurukshetra 132119, Haryana, India.

Male sterility in two allelic single recessive gene mutants designated as *msg3c* and *msg3d* is complete and stable, the female fertility being nomal and unimpaired. In both these mutant lines, the male meiosis is staggered and punctured and the male sterile (ms) gene action is diffuse and continuous. Whereas in *msg3c* , the phasic and periodic breakdown followed by PMC degeneration occurs during zygotene, diplotene, diakinesi AI and TII,in the *msg3d* mutant, the gene action is earlier as the breakdown and degeneration of some PMCs occurs during premeiosis.This is followed by leptotene, zygotene, pachytene, diplotene, metaphase I and anaphase I breakdown of the PMCs. In both these mutant lines, male meiosis continues abnormally till the end in the non-degenerating PMCs (27% and 17% in *msg3c* and *msg3d* respectively); the stepwise meiotic breakdown proportion and amount of anomalies differ significantly in these mutants. Their causes and consequences are discussed.

Keywords: Anther specific genes, Punctured meiosis, Diffuse gene action.

Introduction

Anther development represents a fascinating system to study cell specific gene expression[1-3] as the anther lodges a specialised tissue which after differentiating into spore mother cell undergoes meiosis, a highly coordinated physiological, biochemical, cytogenetical and phenotypic event leading to gene recombination, chromosome reduction and gamete formation[4]. The meiosis involves a precisely regulated and delicately balanced genetically programmed events that exhibits harmonious combination of universality and uniqueness[5]. Male sterile genes distort this program in anther and result in the non-development of a functional anther which is either unable to produce or release viable pollen. The action sequence of each male sterile gene whether nuclear (*ms*) or cytoplasmic (*fr*) is sex-., site-, time-, type-, and target-, specific. Accordingly, the alleles of both *ms* and *fr* genes should not exhibit any meiotic diversion from their gene specificity. But this diversion has been detected in the two alleles of male sterile lines of *Pisum sativum* whose nuclear *ms* genes exhibit allelic identity to the *msg3* gene. These two genetic lines and their respective *ms* genes have been presently designated as *msg3c* and *msg3d*. Their microsporogenesis though deterministic to reach the end meiotic stage culminates before microspore maturity and is described in this paper.

Material and Methods

The mutants were isolated from a segregating population of Bonneville pea variety whose 4 hr presoaked seeds were irradiated with 10kR γ–rays and another similar seed lot treated with 0.1 ethyl methane sulphonate solution for 4 hrs followed by thorough washing in running tap water.The mutagen treated seeds were sown to raise M_1 generation whose seeds on selfing produced M_2 generation. The mutants were identified by their white soft shrunken pollenless anthers at anthesis, delayed flowering and senescence. They were crossed artificially by pollinating with fertile pollen of the untreated mother variety. Selfing M_2 produced seeds to yield M_3 generation. Subsequently M_4-M_6 generations were raised. The selfs, crosses and backcrosses indicated monogenic recessive control of male sterility in these two mutants. Allelism tests revealed

their genes allelic to *msg3c* and *msg3d* and these therefore were designated as *msg3c* and *msg3d*, respectively.

For meiotic studies, the flower buds were fixed for 48 hrs during November-December between 10-11 a.m. (temperature range 14-20° C day, 4-9° C night) in freshly prepared acetic alcohol (1:3), preserved in 70% ethanol and squashed in 1% acetocarmine. The major meiotic events were quantified (Table 1).

Observations

In both these male sterile pea mutants, meiotic breakdown is sequential, progressive and periodic (Table 1), so that only about 15-27% PMCs reach the end stage of meiosis to produce microspores. Despite the microspore production, the microspores do not mature but degenerate gradually during development. Consequently, no fertile pollen grains are produced by both these mutants. Thus their male meiosis though deterministic and degenerative, is unable to produce any fertile pollen despite completion. This meiosis is briefly described in the following, the data are quantified in Table 1 and the major events are depicted by Figs.1-22. To avoid repetition, descriptions of figures are not given in the text.

In both these male sterile genetic lines, the meiosis progresses but the PMC number/anther undergoing meiosis diminishes as in those in which male meiosis breaks down, cytoplasmic and chromosomal disintegration follows their whole cell degeneration. Unlike in *msg3c*, the premeiotic breakdown is absent in *msg3d* (Table1). In leptotene-zygotene, dys-synapsis and chromosomes fragmentations, diplotene and AI breakdown, both these mutants resemble each other. But the diakinesis and TII breakdown of PMCs of *msg3c* mutant are absent in *msg3d*; reverse is true for the pachytene and MII (Table 1). The number of PMCs exhibiting breakdown at MI in *msg3d* significantly exceeds to those of *msg3c*. All the above mentioned differences between *msg3c* and *msg3d* in the proportion of PMCs breakdown during various meiotic stages induce considerable differences (over 12%), in the total proportion of meiocytes which continue meiotic progression till to end.

Discussion

Of the various known types of mutant genes affecting microsporogenesis, the most prevalent in plant kingdom are the mutant genes disrupting normal androecial development form and/or function that leads to male sterility[5]. These mutant genes are either solely nuclear or cytoplasmic or both leading to genic (nuclear), cytoplasmic (plasmatic) and gene-cytoplasmic (nucleo-plasmic) male sterility, respectively. In *Pisum sativum*, only the nuclear genes controlling male sterility are known[3,5-15]. Though majority of these mutant genes are male sex-, site-, stage-and sequence-specific, in some mutants of *Pisum sativum* this specificity is obviated. The time-, stage- and site- of the *ms* gene action over microsporogenesis is diverse and diffuse[10,16]. Consequently, an array of meiotic anomalies and divergently different mode of gene action are evident in those mutants. For instance, the *ms* genes disrupt pre- and post-meiosis[11] induce dys-synapsis[9] or act during heterotypic or homotypic division[13] or exhibit duplicity in gene action[14]. However, in all these mutants, the *ms* gene action is distinctly defined and pronounced. But in *msg3c* and *msg3d* it is awry and punctured. It is difficult to define or delimit the precise mode of *ms* gene action as the gene appears to act strategically during almost all the major meiotic stages (Table 1).

The earliest *ms* gene action occurs in *msg3d* in which the gene acts during premeiosis resulting in pre-meiotic cell death

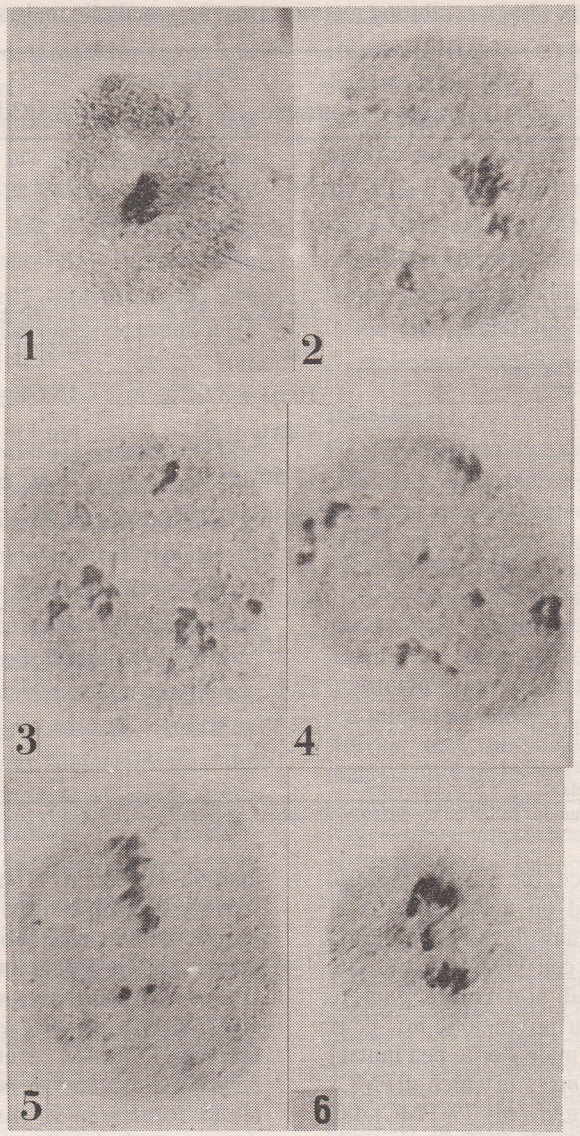
Table 1. Proportion of PMCs exhibiting meiotic arrest in two allelic male sterile pea mutants.

	Stage of arrest	PMC percent msg3c	msg3d
1.	*Premeiosis*	Nil	7.32 ± 1.03
2.	*Meiosis I*		
	Prophase I		
	a) Leptotene & Zygotene	13.18 ± 2.35 a	12.95 ± 3.17a
	b) Pachytene	Nil	9.57 ± 1.26
	c) Diplotene	16.o1 ± 3.92 a	15.64 ± 3.16 a
	d) Diakinesis	5.43 ± 1.28	Nil
	Metaphase I	14.56 ± 3.91 a	22.07 ± 4.35 b
	Anaphase I	10.33 ± 2.19 a	9.92 ± 2.47 a
3.	*Meiosis II*		
	MetaphaseII	Nil	8.17 ± 2.45
	Telophase II	13.16 ± 2.45	Nil
4.	Meiosis arrested	72.67 ± 2.08	85.64 ± 2.27
5.	Meiosis continued	27.33 ± 3.14	14.36 ± 3.05

N =400 (40 PMCs X 10 plants), ± = Standard error.
Mean values of the two mutants followed by different alphabets (a, b) differ significantly from each other at 5P level.

in 7% PMCs. Since the whole meiotic architecture is based on the occurence of normal premeiosis, any disturbance during this division leads to cell death and/or erratic meiotic course and non-viable gamete production[3-5]. Similar pre-meiotic *ms* gene action occurs in the male sterile pea mutant *msg1*[11]. But unlike in *msg1* in which all PMCs degenerate, in the *msg3d*, only 7% PMCs die, in the remaining the male meiosis proceeds erratically. Gradually, the gene action becomes lethal so that PMCs degenerate in phases during subsequent meiotic stages. Thus, only 27% PMCs in *msg3c* and 14% PMCs in *msg3d* complete meiosis though abnormally and consequently produce non-viable microspores and degenerated pollen.

In the mode of *ms* gene action, the *msg3c* gene resembles *msg3d* in that the meiotic cell lethality is phasic and microspore sterility is complete. But unlike in *msg3d*, in *msg3c*, the *ms* gene action is delayed to early meiosis and no pre-meiotic cell death occurs. However, in both these mutants, 35% in *msg3c* and 46% PMCs in *msg3d* degenerate during prophase I (Table 1). But whereas in *msg3c*, 38% PMCs degenerate between diakinesis to tetrad formation, in *msg3d*, 40% PMCs degenerate during these stages. In total, 73-86% PMCs degenerate before microscope formation. Likewise, in both these mutants, the meiotic inhibition is gradual, continuous and phasic. Existence of such a myraidic action of *ms* genes within and between the PMCs of a male sterile mutant is known only in one mutant viz. *msg3* (= *msg3a*) of *Pisum sativum*[12]. In this mutant, in 19,21, 6 and 20% PMCs, the male meiotic inhibition is followed



Male sterile mutant msg3c (Figs. 1-10)

Fig.1 Leptotene stage breakdown; chromatin compact and reduced.

Fig.2 Zygotene stage breakdown; chromatin fragmenting and distancing.

Fig.3-4 Diplotene stage breakdown. The bivalents are diffuse and widely scattered with one showing precocious separation.

Fig.5 Laterally shifted metaphase I plate. One distantly located bivalent separates precociously.

Fig.6 Non-alligned metaphase I chromosomes widely scattered.

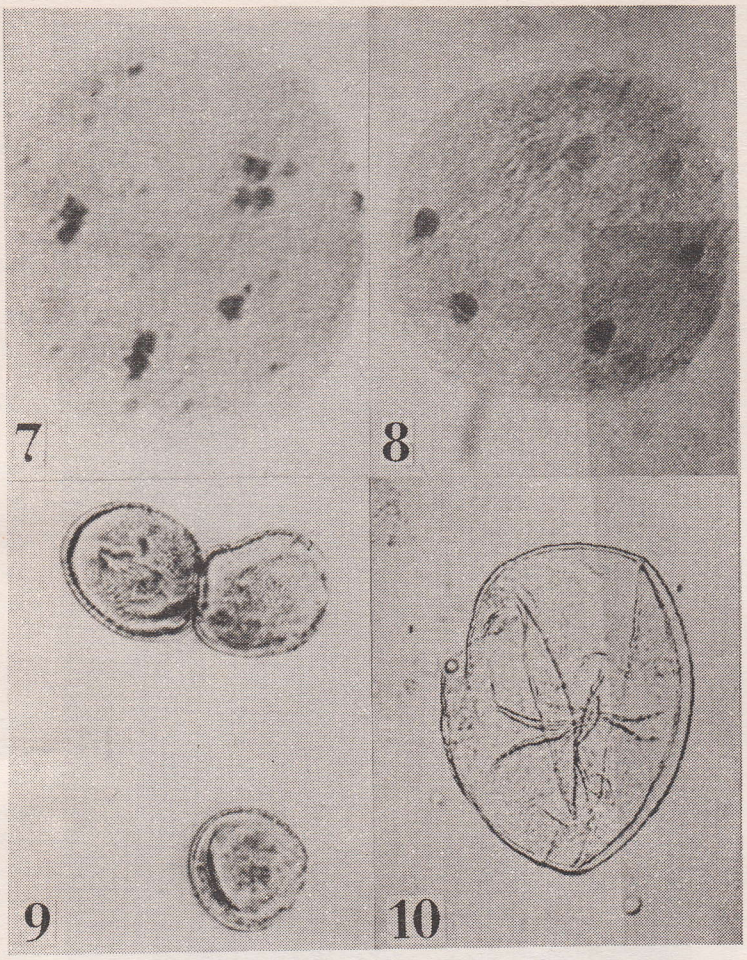


Fig.7 Anaphase I exhibiting unequal chromosomal disjunction and delayed chromosome drag.

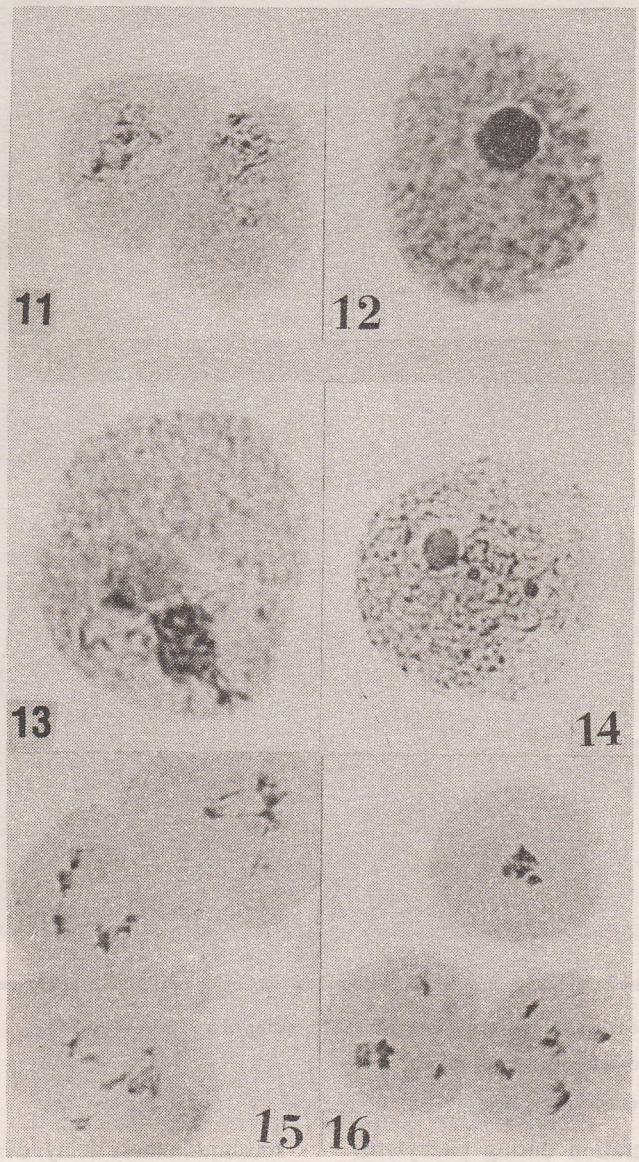
Fig.8 Multiple telophase II nuclei resulting in the formation of a coenocyte.

Fig.9 Young developing microspores.

Fig.10 Degenerated microspores.

by PMC degeneration during pachytene, diplotene, prophase II and telophase II, respectively. But in the mutants *msg3c* and *msg3d*, the gene action spectrum is greatly widened as the mutant genes act almost throughout the microsporogenesis course (Table 1). Why do these differences occur between these three allelic mutants in the time and type of *ms* gene action is not known. Moreover, how and why do some PMCs succumb, others delay, evade or endure the effect of the post-translational protein product of the *ms* genes remains a genetic enigma.

That meiosis is a gene controlled process which requires the correct spatial and temporal expression of many genes is evidenced by detection of many mutant genes influencing meiosis[4,5,17,18]. But how does mutation of a single fertility gene derail the



Male sterile mutant msg3d (Figs. 11-12)

Fig. 11 Premeiotic breakdown; nucleolar degeneration followed by chromatin disruption.

Fig.12 Nuclear compaction followed by disintegration at leptotene.

Fig. 13 Nucleolar followed by compact chromosome degeneration during zygotene.

Fig.14 Pachytene chromosome degeneration accompanied by nucleolar fragmentations.

Fig.15-16 Diplotene bivalent degeneration preceded by nucleolar disintegration.

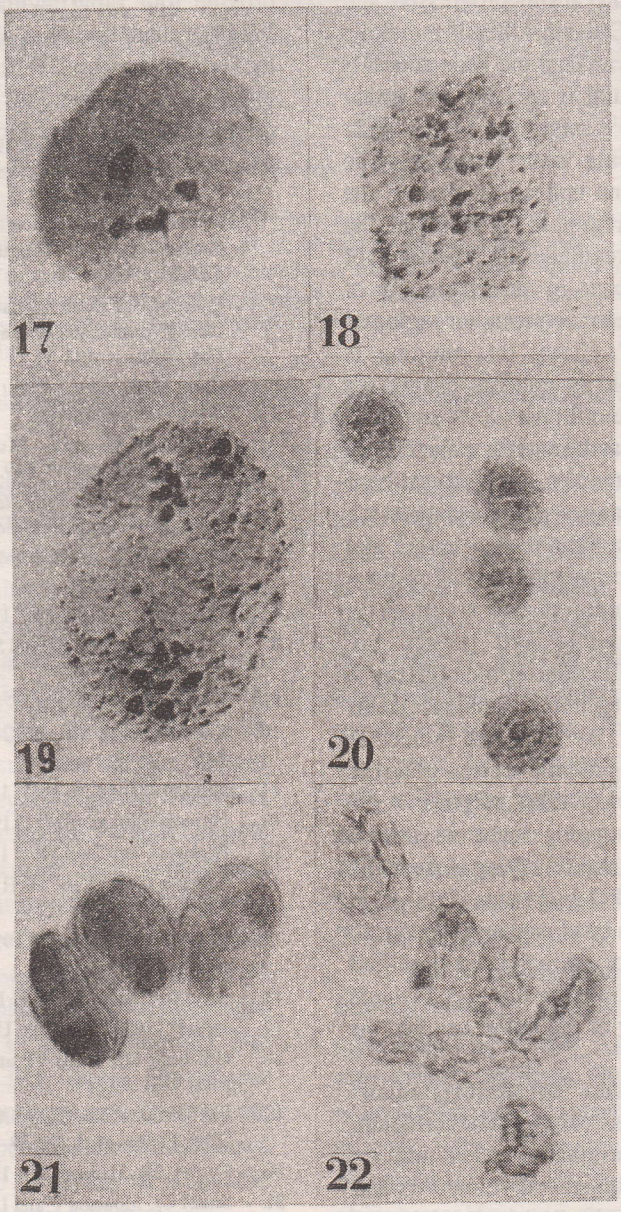


Fig. 17 Metaphase I bivalent clumping.
Fig. 18 Unequal and erratic AI disjunction.
Fig.19 Asynchronous and assymmetric Metaphase II.
Fig. 20 Unequal sized young microspores.
Fig. 21-22 Degenerating (21) and degenerated (22) microspores.

meiotic progression causing progressive and programmed PMC death following meiotic arrest is not known. That the gene action is temporal and spatial is true for the anther specific genes[5,12,18], majority of the male sterility genes being anther specific[3]. The *msg3* mutant gene appears to disturb the highly conserved region in PB promoter, the "anther box" that regulates the expression of anther specific genes by direct binding of their gene product to the promotor region of the target gene[19]. Either this binding is defective or weak as meiosis progresses despite periodic meiotic breakdown and PMC death. It appears that the nuclear male fertility gene mutations invokes tissue-specific or stage- specific changes in gene expression known for CMS mutations in petunia[20] and sunflower[21,22] and in *msg3* mutant of *P.sativum* . Does the mutant gene remain in conflict with the parental genome and induce the evolutionary novelities during meiotic course because of the latent genomic turbulence needs to be investigated. By using the molecular markers wihin the genomic regions of fertile and male sterile *ms* lines of peas, *msg3* targetted genetic mapping can be possible by identifying RAPD markers lying close to the *ms* gene. The RAPD fragments if linked to the *msg3* gene can be cloned and sequenced to develop sequence characterised amplified regions. This will form the first step towards map based cloning of the *msg3* or other *ms* genes of pea.

Despite severe constraints, abnormalities and progressively periodic cell death, the PMCs of both the mutants are deterministic to follow through degenerative meiosis and some of these reach microspore development stage but none progresses beyond. The differentiation of premeiotic cells, meiosis, gametophyte development and gametogenesis provide four rapid switches in cell fate[23]. Whether these switches are programmed in the microsporogenous cells that compells them to follow the pathway till it is erased due to premature microspore abortion in these two pea mutants is not known.

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