Male Sterility in Pea I. Genes disrupting pre- and post-meiosis

C. Nirmala and M. L. H. Kaul

Department of Botany, Kurukshetra University, Kurukshetra-132 119 (Haryana), India

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Three distinct sequential stages comprise the microsporogenesis. These are: the pre-meiosis, meiosis and post-meiosis. Pre-meiosis is the preparatory stage of a meiocyte to undergo meiosis. During this stage, the cells separate from one another attaining thereby the genetic autonomy, secretes a callose wall and synthesises about 96% DNA (Kaul 1988). This is followed by meiosis that involves a series of sensitive, specific and specialized events leading to microspore production. Once the microspores are liberated from PMCs, they enter post-meiosis—a preparatory stage for gamete production. During this stage, the microspores separate from each other, secrete a callose wall and prepare for a mitotic division. All these events in the microspore comprise post-meiosis. Of all these three distinct development stages, the meiosis is a long duration, time and energy consuming cellular event. It is controlled and co-ordinated by a large number of genes majority of which are dominant (Gottschalk and Kaul 1974, Kaul and Murthy 1985). Compared to the meiotic events, both pre- and post-meiotic events are controlled by a relatively fewer number of genes. Despite this fewer number of gene control, majority of the mutant genes inducing male sterility are post-meiotic (Kaul 1988, Kaul and Nirmala 1989). Whereas reasons of this preferential mutant gene action are not known, the net effect of all the ms genes is induction of male sterility accompanied by unimpaired female fertility.

Of the eight male sterile mutants induced in Pisum sativum, the ms gene in one mutant acts during pre-meiosis thereby obviating meiosis and in two mutants, the genes act during post-meiosis preceded by normal meiosis. In the remaining five mutants, the male meiosis is impaired by the ms genes. Causes and consequences of the pre-meiotic and post-meiotic ms genes in Pisum sativum comprises the text of this paper.

Material and methods

Mutant isolation

Three male sterile mutants, msg-1, msg-7 and msg-8 were obtained by subjecting seeds of two Indian pea varieties Arkel and Bonneville to physical and chemical mutagens. Their seeds were pre-soaked in distilled water for 12 hr. The mutant msg-1 was induced by a combinational treatment of Bonneville by 5 kR (50 Sv) gamma rays followed by 4 hr soaking in 0.05% ethyl methane sulphonate. The mutants msg-7 and msg-8 were obtained by treating Arkel for 4 hr in 0.10% ethyl methane sulphonate and diethyl sulphonate, respectively. From the treated seeds, M1 progeny was raised whose selfed seeds produced the M2 generation. Segregations for male sterility and fertility and their subsequent chi-square analysis indicated the sterility to be controlled by single recessive genes. Tests for allelism using heterozygote fertiles in direct and reciprocal crosses indicated the three ms genes as non-allelic.

Sample size

Twenty flower buds each from 20 male sterile plants per genotype were used for meiotic

1 Corresponding author.
investigations. In total, three generation plants were utilized. Thus, 1,200 floral buds from 60 male sterile plants were examined cytologically.

**Meiotic studies**

For this, the flower buds were collected from both male sterile and fertile plants between 10–11 a.m. and fixed in freshly prepared acetic alcohol (1:3). After 24 hr of fixation, the material was washed thoroughly with water and preserved in 70% ethanol. The anthers were squashed in 1% acetocarmine.

<table>
<thead>
<tr>
<th>Table 1. Main cytological events in mutant msg-1</th>
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<td>Cytological event</td>
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| 1) Early formed flowers (0–7%)  
PMCs do not differentiate, the cells degenerate | 4±2.81  | 100 |
| 2) Normally formed flowers (91–100%)  
PMCs differentiate, separate out and degenerate | 94±5.73  | 100 |

SD = standard deviation  
*: the absolute values are in terms of the major cytological events.

Figs. 1–3. (Mutant msg-1). 1, Cytoplasmic amalgamation of PMCs. 2, Clumping of chromatin material. 3, Nucleolar and chromatin clumps moving to the periphery.

**Observations**

**Pre-meiotic mutant**

*Mutant msg-1*: Floral buds formed 6–9 days prior to the main flowering flush are termed as the early formed flowers. In this mutant, early formed buds comprise 4% of the total buds produced by the plant (Table 1). The PMCs do not attain autonomy, separation and independence as the *ms* genes inhibit the wall formation. The cytoplasm of the PMCs amalgamate into one another (Fig. 1). The switch on for the meiotic initiation does not occur in such PMCs. Their chromatin clumps and no further division stages follow (Fig. 2). Thus, all the PMCs of the early formed few flowers degenerate without undergoing meiosis.

Majority of the flowers (94%) of this mutant are produced during the normal flowering period (November-December). In them, the PMCs differentiate and separate out from one
another but no callose wall is formed around each PMC. The chromatin material condenses to form a compact ball (Fig. 3). The nucleolus is also highly compact and either remains near the chromatin ball or distances away from it. Thus the mutant is ameiotic, pollenless and hence completely male sterile.

Figs. 4–11. (Mutant msg-7). 4, Monads in free state. 5, Monads in adhered state. 6, Nuclear fragmentation in young microspores. 7, 8, Nuclear and cytoplasmic degeneration in microspores. 9, Cytoplasmic degeneration preceding nuclear degeneration. 10, 11, Cytoplasmic and chromatin condensation.
Post-meiotic mutants

Mutant msg-7: In this mutant, 2–4 flowers per plants are developed at 3–4th basal nodes, five to six days before normal flowering. These flowers abort and drop off without anther or carpel formation. Therefore, the buds investigated in this mutant were collected during the peak flowering period viz. November-December. In these buds, the male meiosis is normal until tetrad formation. While in 87% PMCs, the monads separate out from one another (Fig. 4), in 13% the monads remain in an adhered state (Fig. 5). Nuclei of the monads fragment resulting in the formation of a large number of nuclei (Fig. 6). Gradually nuclei degenerate along with the cytoplasm (Figs. 7, 8). In 26% of the freed microspores, chromatin fragmentation occurs (Table 2). In them the cytoplasmic degeneration is more rapid than the chromatin degeneration (Fig. 9). In 74% microspores, the chromatin condensation is rampant. The microspore walls become abnormally thickened giving appearance of a wall within a wall. In

<table>
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<tr>
<th>Cytological events</th>
<th>PMC (%)</th>
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<td>Mean ± SD</td>
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<td>A) Microspores released:</td>
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<tr>
<td>i) in free state</td>
<td>87 ± 9.75</td>
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<tr>
<td>ii) in adhered state</td>
<td>13 ± 4.28</td>
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<tr>
<td>B) Microspore degeneration:</td>
<td></td>
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<tr>
<td>i) chromatin fragmentation coupled with cytoplasm disintegration in monads</td>
<td>26 ± 7.91</td>
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<tr>
<td>ii) chromatin condensation and abnormally thickened microspore wall formation</td>
<td>74 ± 12.57</td>
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Table 3. Main cytological events in mutant msg-8

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<th>Cytological events</th>
<th>PMCs (%)</th>
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<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
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<tr>
<td>A) Microspores released (100%)</td>
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<tr>
<td>B) Degeneration of microspores (100%)</td>
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</tr>
<tr>
<td>i) nuclear degeneration faster than cytoplasmic degeneration</td>
<td>93 ± 5.88</td>
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<tr>
<td>ii) cytoplasmic degeneration faster than nuclear degeneration</td>
<td>7 ± 4.95</td>
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SD: standard deviation
(Absolute values are the same as the mean percent values)

all such thick walled microspores, the cytoplasm and chromatin material aggregate and condense forming a continuous or a discontinuous peripheral vacuole (Figs. 10, 11). Gradually the chromatin and cytoplasm of all the microspores shrivel and degenerate completely. Thus no pollen are formed in this mutant. Hence it is completely male sterile.

Mutant msg-8: As in msg-7, in this mutant also, 1–3 buds per plant are developed at 3–4th basal nodes four to five days prior to the normal flowering period. But they drop off prematurely before differentiation of stamens and carpels. In the flowers formed during the main flowering flush, the male meiosis proceeds normally until tetrad formation. Monads are released and these develop a thin double-layered wall. The nuclear chromatin undergoes extreme condensation after it migrates from the peripheral to the central position of the microspore (Fig. 12). This results in the formation of a peripheral vacuole with or without a fragmented chromatin giving the appearance of a micronuclei in the former case (Fig. 13). No microspore development occurs but their chromatin material gradually disintegrates along with the cyto-
plasm. In 93% microspores, the cytoplasm degeneration is more rapid than the nuclear degeneration (Fig. 14). Only in 7% microspores, the nuclear degeneration is slower than the cytoplasmic degeneration (Table 3). Due to degeneration of the chromatin and cytoplasm, the vacuolar size increases in each microspore (Figs. 15, 16). Finally, the microspores degenerate and exhibit a tendency to clump with each other. Large number of microspores occur in a clumped mass exhibiting various stages of cytoplasmic and chromatin degeneration. No viable pollen grains are produced by this mutant. Hence like msg-7, this mutant is completely male sterile.

**Discussion**

The whole meiotic architecture is based on the occurrence of normal pre-meiosis as any disturbance during this division leads to erratic meiotic course and non-viable gamete production (Gottschalk and Kaul 1974, Baker et al. 1976, Golubovskaya 1979, Kaul and Murthy 1985). In the PMCs or MMCs, the meiotic processes start only after the successful and normal culmination of this initial event. Premeiotic ms gene action in male sterile mutants is documented in Capsicum annuum (Hirose 1965), Cirsium oleraceum (Delannay 1979), Citrus aur-
antifolia (Uphof 1931, Nakamura 1934, Krug and Bacchi 1943), C. unshiu (Osawa 1912, Nagai and Tanikawa 1928), Cucurbita maxima (Rhodes 1959, Singh and Rhodes 1961), Fagopyrum esculentum, Geranium argenteum, Hordeum vulgare (Kaul 1988), Limnanthes douglasii (Jain et al. 1978), Lycopersicon esculentum (Rick and Butler 1956, Zamir et al. 1980, Kaul 1988), Oenothera gigas (Gates 1911), Pismum sativum (Gottschalk 1961, Gottschalk and Kaul 1974, Klein and Milutinovic 1971, Kaul 1988), Rubus idaeus (Crane and Thomas 1949), Solanum tuberosum (Breeze 1921, Fukuda 1927, Heyn 1930), S. vulgare (Ayyangar and Ponnaiya 1937, Stephens 1937) and Zea mays (Beadle 1932, Palmer 1971, Albertsen and Phillips 1981). In all these, the ms genes either inhibit the development and differentiation of anthers or archesporial tissue and/or suppress PMC development and differentiation. In them since PMCs are either not developed or abnormally formed, they neither exhibit pre-meiosis nor have tendency to enter into meiosis. Therefore all such instances of ms gene action are not pre-meiotic.

In legumes, male sterility is documented in 24 species and gene action is determined for 60 cases (Kaul 1988). Of these, the action of ms gene in one mutant of Lathyrus odoratus and in three mutants of Pismum sativum is considered as pre-meiotic by Faberge (1937), Klein and Milutinovic (1971) and Kaul (1988). In L. odoratus, though archesporial cells are differentiated, PMCs are not developed. Whereas in one gamma ray induced mutant of P. sativum, the anthers are not developed (Gottschalk 1961), in another induced mutant, meiocytes are not formed (Kaul 1988). In the neutron induced mutant, no archesporial tissue is developed (Klein and Milutinovic 1971). Therefore, all these male sterile mutants are not pre-meiotic mutants.

The first instance of a male sterile mutant gene acting during pre-meiosis is documented presently in the Pismum sativum mutant msg-1. In it, the anther development and PMC differentiation occurs normally. The PMCs separate out from one another and prepare for pre-meiosis. But gradually, their chromatin and nucleolus degenerate and they do not develop a callose wall. Thus whereas the gene action is pre-meiotic, the mutant is ameiotic functionally.

Microspore formation, the first main event after meiotic completion, is a crucial post-meiotic stage as it is the stage most susceptible to ms gene action as majority of the post-meiotic genes act just after the microspores are formed and released from the quartet (Kaul 1988). This is evidenced in legumes as well because of the 60 investigated cases, in 22 the ms gene action is post-meiotic (Kaul 1988). Of the 8 male sterile mutants investigated presently in P. sativum, 2 are post-meiotic viz. msg-7 and msg-8. In msg-7, whereas in 87% of PMCs, the microspores are released in free state, in 13% they stay in adhered manner. Thereafter, either chromatin fragmentation or condensation occurs in the microspores and they gradually abort. Whether the penetrance of msg-7 gene is variable or the gene action is delayed or majority of the microspores escape the gene action through quick release from the anther sac could not be determined. But that the ms gene action is operational during post-meiosis is evidenced by abortion of all the microspores whether free or fused in the anthers of the mutant. A delayed ms gene action occurs in the mutant msg-8. In this mutant, the male meiosis is normal and microspores are released. But just after release, they degenerate through nuclear degeneration which precedes or follows cytoplasmic degeneration. Further delay in the ms gene action occurs in mutant 503 studied by Klein (1969). The ms gene acts during pollen development and inhibits exine and aperture formation. Since the mutants msg-8 and 503 exhibit a nearly similar gene action, the difference being in the timing, it is worthwhile to test their genetic identity. Delay or hastening of ms gene action is influenced by the environmental factors like temperature and photoperiod (Kaul 1988, Polowick and Sawhney 1990, 1991). Therefore coupled with allelism tests, it is feasible to grow both these mutants under one set of environmental conditions and observe the time and consequences of ms gene action.
Conclusions

From the above, it is concluded that the time and mode of ms gene action in the three P. sativum male sterile mutants, msg-1, msg-7 and msg-8, does not alter the degree or expression of male sterility or female fertility. Both of these are complete and uniform in all the three mutants. Moreover, whatever are the causes of pollen sterility in P. sativum, the consequences are similar viz., complete male sterility. Does this hold true for other Pisum male sterile mutants as well remains to be investigated. Are these generalisations valid only for the anther specific genes or also for the ovule specific genes? Both these gene types are known in higher plants (Kaul 1988). Of these two, the mRNAs and cDNAs for the anther specific genes in tobacco (Goldberg 1988) and tomato (Ursin et al. 1989, McCormick et al. 1989) and pollen specific genes in Zea mays and Tradescantia (Mascarenhas 1988, Hanson et al. 1989) have been isolated. A chimeric ribonuclease gene within an anther selectively destroys the tapetal cells, prevents pollen formation and leads to male sterility in transgenic tobacco and oilseed rape plants (Mariani et al. 1990). Can this or similar anther specific gene induce male sterility in pea if it is made transgenic? Transgenic pea has just been developed by Kathen and Jacobsen (1990) and Pounti-Kaerlas et al. (1990).

Summary

Following EMS, DES and gamma ray treatment in Arkel and Bonneville pea varieties, three male sterile mutants were induced in Pisum sativum. Sterility in each of these is conditioned by single recessive genes, the three genes being non-allelic. Whereas in one mutant, the ms gene acts during pre-meiosis, in the other two the genes act during post-meiosis. In the pre-meiotic mutant, the PMCs either degenerate before attaining genetic autonomy or after separating out from one another. Their chromatin either compacts or fragments to degenerate completely. Thus, no meiosis occurs in this mutant.

In both the post-meiotic mutants, male meiosis is normal. In one, the microspores are released from the PMCs either in free or in adhered state. They develop thick walls, their chromatin condenses centrally and finally they degenerate. In the other mutant, monads are released from the PMCs. In 93% microspores, the nuclear degeneration is faster than cytoplasmic degeneration, in 7% the reverse occurs. In both the post-meiotic mutants, microspores degenerate fully. Male sterility in all the three mutants is complete while female fertility is normal.

Acknowledgements

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References


