POLLEN ABORTION IN *SESAMUM INDICUM* L. PLANTS TREATED WITH GAMETOCIDES*

S. V. S. CHAUHAN and Toshiro KINOSHITA**

*Department of Botany, R. B. S. College, Agra India

**Plant Breeding Institute, Faculty of Agriculture, Hokkaido University, Sapporo 060 Japan

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Introduction

Induction of male sterility in plants through gametocidal compounds in recent years has drawn considerable attention (see Mohan RAM and RUSTAGI[14], DUBEY and SINGH[8], KINOSHITA[13], and CHAUHAN and SINGH[5]. However, our understanding on the mode of action of these compounds on anther development is meager. The present paper deals with morphological and histochemical studies in *Sesamum indicum* plants treated with some gametocides.

Materials and Methods

The seeds of *Sesamum indicum* var. T. 4 were sown in the Botainc Gardens, R. B. S. College, Agra and the plants thus raised were sprayed with aqueous solutions of 0.25, 0.5 & 1% Maleic hydrazide (1, 2-dihydropyridiazine 3, 5-dione); 0.15, 0.3 & 0.5% FW-450 (sodium 2, 3-dichloroisobutyrate) and 0.1, 0.25 & 0.5% Dalapon (2, 2-dichloropropionic acid). Some plants were treated only once at prefloral bud initiation stage (T_1), some others were treated twice at both pre-and post-floral bud initiation stages (T_2) and still others were treated three times at pre-and post-floral bud initiation stages and at the time of anthesis (T_3). A few plants were sprayed with distilled water to serve as controls (T_0).

Pollen viability of treated and control plants was checked at regular intervals by the method developed by Alexander[2]. Flower buds of these plants were fixed in formalin-acetic-alocohol and 80% acetone for morphological and histochemical studies respectively. These were dehydrated, cleared and embedded by customary procedures. Sections were cut at 7–12 μ. For

the histochemical procedures described by JENSEN[10] for localization of total proteins (Ninhydrin-schiff's reaction), total carbohydrates of insoluble polysaccharides (PAS test), DNA (Feulgen reaction) and histones (Akaline-fast green test) in the microtomes sections were followed.

Results and Discussion

The effect of various gametocides on growth, flowering, yield and pollen viability has been published elesewhere[4,5]. Based on the extent of pollen sterility induced through various gametocidal treatments, the treated plants were classified into four groups, namely; normal (N), semi-sterile a (S. S. a), semi-sterile b (S. S. b) and complete sterile (C. S.). The percentage of pollen sterility and dehiscent and non-dehiscent nature of anthers in various treatments are given in Table 1.

TABLE 1. Classification of chemically treated plants

Type	Control Cont.	Normal N	Semi-sterile a S. S. a	Semi-sterile b S. S. b	Complete sterile C. S.
Concentration & application of gametocides	T_0	Dalapon 0.1% (T_1)	0.1% (T_2, T_3)	0.25% (T_3)	0.5% (T_3)
		0.25% (T_1)	0.25% (T_2)	0.5% (T_2)	
			0.5% (T_1)		
		FW-450 0.15% (T_1)	0.15% (T_2)	0.15% (T_3)	0.5% (T_3)
		0.3% (T_1)	0.3% (T_2)	0.3% (T_3)	
			0.5% (T_1, T_2)		
		MH* 0.25% (T_1)	0.25% (T_2)	0.25% (T_3)	1.0% (T_3)
				0.5% (T_1, T_2)	
				1.0% (T_1, T_2)	
Pollen sterility (%)	0–10	0–10	11–50	51–95	96–100
Dehiscent	+	+	+	±	−

* MH: Maleic hydrazide

Morphological observations

The anther wall layer at sporogenous tissue stage consisted of an epidermis, 1-2 layers of endothecium, 2-3 middle layers and a glandular tapetum. Development of anther in N and S. S. a type of plants was similar to that of non-treated (control) plants (Fig. 1). However, the degeneration of tapetum in the anthers of S. S. a type of plants was delayed. The inner tangential walls of tapetum showed the presence of deeply stained refractive droplets

called 'Ubisch bodies'. The anthers in S. S. b type of plants were either dehiscent or non-dehiscent in the same flower at different rates of mixing. Development of dehiscent anthers of this group of plants was similar to that of S. S. a type except that the degeneration of tapetum was further delayed. The walls of tapetal cells in such anthers disintegrated and the protoplasmic contents migrated in the locules among the microspores to make a pseudoperiplasmodium (Fig. 2). The formation of fibrous bands in the endothecial cells of such anthers was either delayed or partially inhibited (Fig. 3). On the other hand, tapetal layer failed to degenerate in the non-dehiscent anthers of S. S. b type of plants. The cells in this layer elongated radially and fibrous bands in the endothecium were absent (Fig. 4). These findings support the programmed control of tapetum on the development of endothecium suggested by De Fossard[7]. According to him, the development of endothecium is inhibited throughout the major course of anther development and only after complete degeneration of tapetum, fibrous thickenings in the endothecial cells develop.

a. FW-450: The anthers of plants treated with 0.5% (T_3) FW-450 showed abnormal enlargement of tapetal cells. The cells in this layer became hypertrophied at vacuolate pollen grain stage (Fig. 5). Development of endothecium in such anthers was inhibited, thus confirming De Fossard's hypothesis[7]. However, in a limited number of anthers, the tapetal cells degenerated much prior to the onset of meiosis in the PMCs. This was followed by the degeneration of spore mother cells and further development of anthers ceased at this stage (Fig. 6).

b. Maleic hydrazide & Dalapon: In the anthers of plants treated with 0.5% (T_3) Dalapon and 1% (T_3) Maleic hydrazide, the tapetal cells enmass migrated into the anther locules and mingled with the pollen grains to make a pseudoperiplasmodium (Fig. 7). At the time of anthesis, pollen grains degenerated along with the protoplasts of the plasmodium (Fig. 8). The formation of fibrous bands in the endothecial cells in such anthers was fully inhibited. Abortion of pollen caused by the pseudoperiplasmodium and tapetal hypertrophy has been recorded in several cytoplasmic, genic as well as chemically induced male sterile plants[9,11,12,13]. According to these papers, the pseudoperiplasmodium of the tapetum in male sterile anthers seems to hinder the development of normal pollen grains. This is largely because the fertile counterparts of such plants exhibit only glandular tapetum. Thus in sterile anthers, formation of periplasmodium was not a source of nutritional supply. Hypertrophy of tapetal cells in C. S. anthers, according to the present authors seems to be a reaction in search of nutrition as suggested earlier by CHAUHAN

and SINGH[3] in genic male-sterile *Cucumis melo*. This is corroborated by the fact that the procambial tissue in the anther connective of C. S. plants failed to differentiate (Fig. 9).

Histochemical observations

In early stages of development, the intensity of various histochemical reactions in different parts of an anther in control and C. S. type of plants was low or moderate. However, in the anthers of control plants, the intensity of various reactions increased with age in different parts, particularly in the tapetum and developing microspores. The most intense reaction by the former tissue was shown at microspore tetrad stage, while the mature pollen grains showed highest concentration of various substances (Figs. 10, 11). On the other hand, the anthers of C. S. type of plants were markedly deficient of these substances at all stages (Fig. 12). Deficiency of total carbohydrates of insoluble polysaccharides, DNA, histones and proteins in the anthers of several cytoplasmic, genic and chemically induced male-sterile plants has been recorded by various workers[1,3,6,15,16]. The inhibition of vascular differentiation in the anthers of C. S. type of plants, in the opinion of the present authers blocks the path of various nutrients to pass into various parts of an anther including the tapetum and finally to developing microspores. Because of starvation, tapetum became malformed and microspores deprived of nutrition aborted.

Summary

Pollen abortion in chemically induced male-sterile plants of *Sesamum indicum* was studied. Various treatments with gametocides at different stages caused pollen sterility of variable degrees. The abortion of pollen was associated with tapetal abnormalities similar to those exhibited by cytoplasmic or genic male-sterile plants. Histochemical studies revealed the deficiency of carbohydrates, proteins, DNA and histones in the anthers of complete male-sterile plants. It is concluded that the inhibition of vascular differentiation in such anthers caused malfunctioning of tapetum and finally the abortion of pollen.

Acknowledgements

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Figs. 1–12. Transverse part of anthers of treated and untreated plants of *Sesamum indicum* L.

Fig. 1. Cont.: Mature anthers. 280×.

Fig. 2. S. S. b: Dehiscent anther showing pseudoperiplasmodium. 280×.

Fig. 3. S. S. b: Dehiscent anther showing pseudoperiplasmodium. 280×.

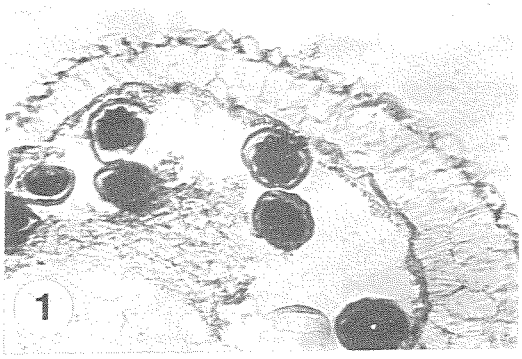
Fig. 4. S. S. b: Non-dehiscent anther showing radially enlarged tapetal cells crushing the microspores. 210×.

Fig. 5. C. S. anther of 0.5% (T_3) FW–450 treated plant showing tapetal hypertrophy. 210×.

Fig. 6. C. S. anther of 0.5% (T_3) FW–450 treated plant showing degeneration of tapetum and PMCs. 210×.

Fig. 7. C. S. anther of 0.5% (T_3) Dalapon treated plant showing pseudoperiplasmodium among the microspores. 210×.

Fig. 8. C. S. anther of 1% (T_3) MH treated plant showing degeneration of plasmodium and microspores. 210×.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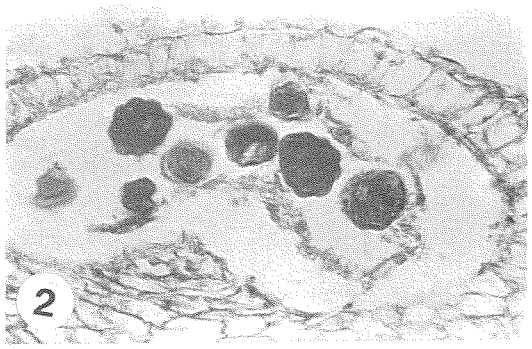
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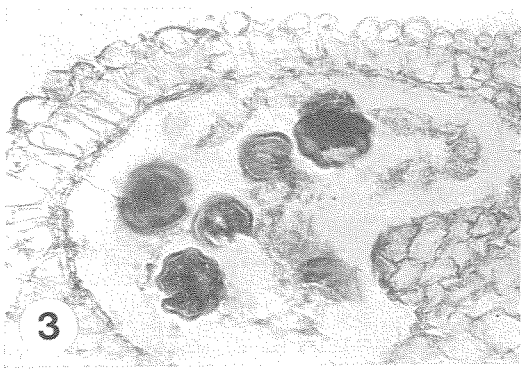
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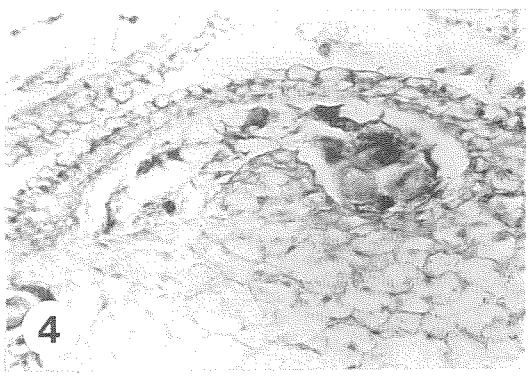
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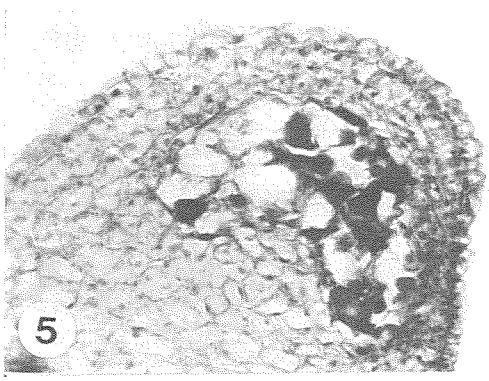
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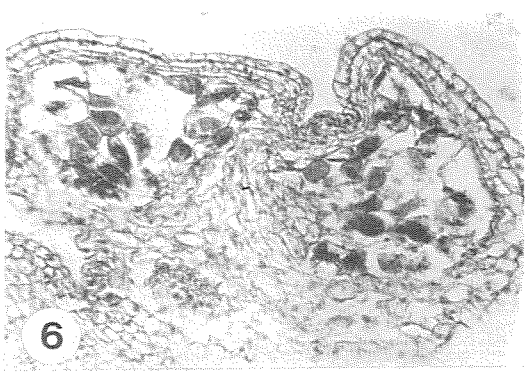
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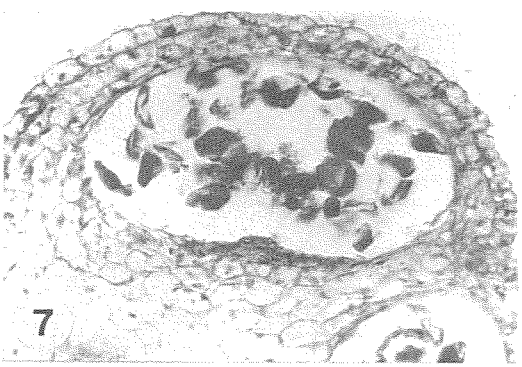
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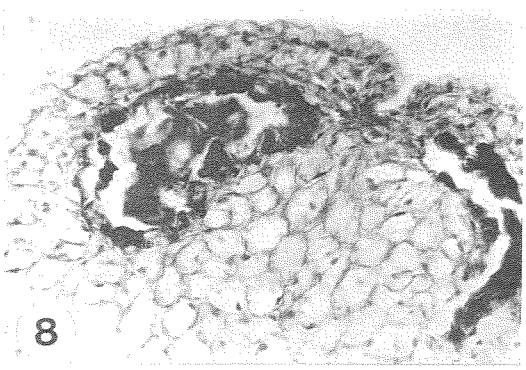
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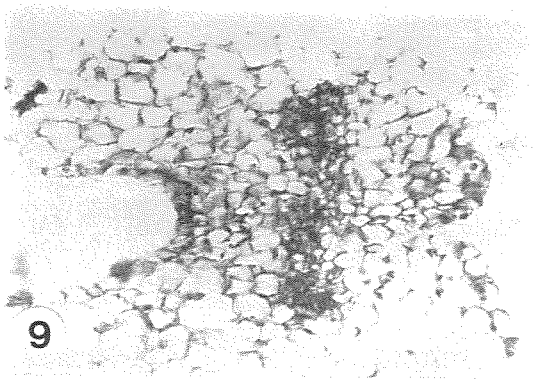
Fig. 9. C. S. mature anther of 1% (T_3) MH treated plant showing undifferentiated pro-cambial strand. 180×.

Fig. 10. Control anther showing PAS reaction. Note the presence of Ubisch bodies on the inner walls of the anther locule and highly PAS positive pollen grains. 280×.

Fig. 11. Control anther showing ninhydrin-schiff's reaction. Note the presence of Ubisch bodies. 280×.

Fig. 12. C. S. anther of 0.5% (T_3) FW-450 plant showing ninhdrin-schiff's reaction at pollen stage. Note the poor reaction in hypertrophied tapetal cells and Ubisch bodies. 210×.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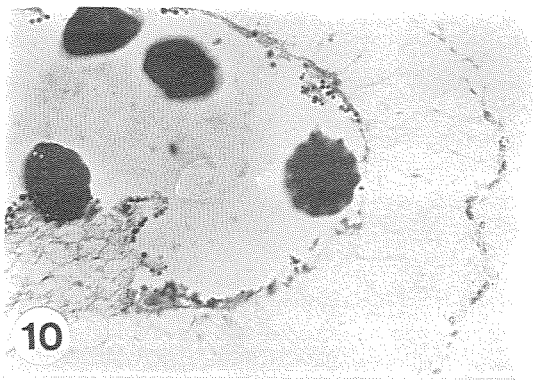
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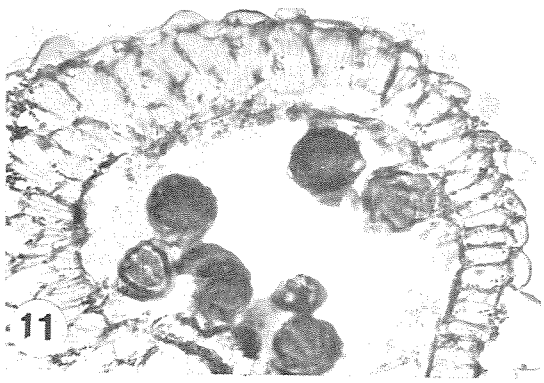
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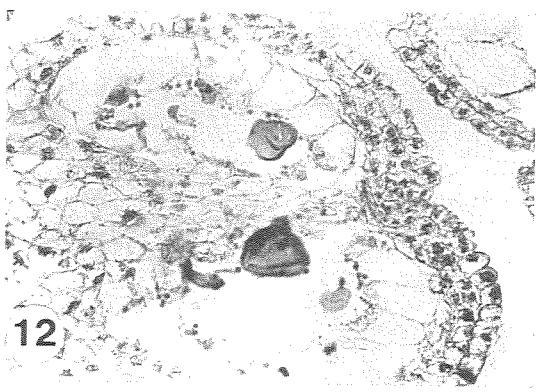
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