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ABNORMAL BEHAVIOUR OF TAPETAL MITOCHONDRIA ASSOCIATED WITH POLLEN ABORTION IN DETERGENT INDUCED MALE STERILE *RAPHANUS SATIVUS* L.

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Foliar applications of 0.5, 1.0 and 1.5% (w/v) surf excel effectively induced 100% pollen sterility in *Raphanus sativus* lasting throughout flowering period. Light microscopic studies revealed that pollen sterility in treated plants was associated with abnormal tapetal behaviour. The cells of the tapetal layer remained intact up to anthesis and were radially enlarged and highly vacuolated. Transmission electron microscopic studies indicated the presence of large number of small thick-walled mitochondria in radially enlarged tapetal cells. Mitochondria degenerated pre-maturely at microspore tetrad stage. This was followed by degeneration of other cell organelles, particularly plastids. The mature pollen grains of treated plants were sterile as was evident by the presence of several degenerated mitochondria in highly vacuolated cytoplasm. Formation of exine, except baculae, was not much influenced but intine at places was abnormally thick.

Key words: anther development/pollen sterility/*Raphanus sativus*/surf-excel

Chemicals capable of selectively inhibiting pollen development and thus blocking male fertility are known as chemical hybridizing agents (CHAs). Chemical induction of male sterility in plants has been of interest since 1950, and so far a large number of chemicals have been tested for induction of male sterility in a large number of angiosperms (Chauhan and Kinoshita 1982, Kaul 1988 and Cross and Schulz 1997). The chemicals inducing male sterility can obviate the lengthy time period required to obtain male sterile and restorer lines, which usually must precede evaluation of hybrid performance. Consequently, chemicals became of interest both for use as breeding tools and as a means of producing hybrid seeds on a commercial scale. Recently, surf excel has been used as a chemical hybridizing agent in some crops e.g. *Vicia faba* (Chauhan and Chauhan, 2003); *Brassica juncea* (Chauhan and Singh, 2002; Singh and Chauhan, 2003); *Nicotiana tabacum* (Agnihotri and Chauhan, 2004); *Abelmoschus esculentus, Capsicum annuum* and *Lycopersicon esculentum* (Chauhan and Agnihotri, 2005) and *Cicer arietinum* (Chauhan and Gupta, 2005).

Numerous anatomical studies conducted primarily with light microscope have established the association of tapetal abnormalities with pollen abortion in plants treated with various CHAs (Chauhan and Kinoshita 1982 and Kaul 1988). However, it is important to find out involvement of a particular tapetal cell organelle in pollen abortion. This can be resolved by ultrastructural studies only (Laser and Lersten 1972). The present investigation highlights the changes in the tapetum and pollen grains of *Raphanus sativus* plants sprayed with detergent surf excel.

MATERIAL AND METHODS

The seeds of *Raphanus sativus* var. Hill Queen obtained from National Seed Corporation, Agra, were sown at the Botanical Garden of the School of Life Sciences, Dr. B. R. Ambedkar University, Agra. The plants raised were sprayed with aqueous solutions of 0.5, 1.0 and 1.5% (w/v) surf excel at three different stages of development. A group of ninety plants was sprayed a week before the initiation of floral buds at pre-meiotic stage (T_1). Leaving a group of thirty plants after first treatment (T_1), the remaining sixty plants were sprayed again at the time of floral bud initiation stage (T_2). After leaving a group of thirty plants receiving two sprays (T_2), the

other thirty plants were again sprayed for the third time, at the time of anthesis (T_3). A group of another ninety plants was sprayed with distilled water (T_0) to serve as control. Pollen fertility was tested at regular intervals with 1% TTC (Tetrazolium Chloride) in 0.15M tris HCL buffer at pH 7.8.

For light microscopic studies, the floral buds of treated plants showing 100% pollen sterility and control plants were fixed in formalin-acetic-alcohal. These were dehydrated and embedded in paraffin by customary methods. Sections were cut at 5-12 μm and stained with Delafield haematoxylin.

For transmission electron microscopy, anthers of treated and control plants at various developmental stages were fixed in 3% glutaraldehyde in 0.1 M PO_4 buffer at pH 6.8. Post fixation was done in 1% Osmic acid in the same buffer. Samples were dehydrated in an ethyl propylene oxide series and embedded in spurr's low viscosity embedding media. Ultrathin sections were cut, stained with uranyl acetate and lead citrate and observed under Phillips Cryo CM-10 electron microscope at All India Institute of Medical Sciences, New Delhi.

RESULTS AND DISCUSSION

Pollen sterility : Foliar applications of different concentrations of surf excel effectively induced pollen sterility ranging between 95.1-100%. Two or three treatments with 1.0% and 1.5% solutions brought about complete pollen sterility lasting throughout the flowering.

Anther development : At sporogenous tissue stage, anther wall in both treated and control plants consisted of an epidermis, 2-3 layers of endothecium, a middle layer and a tapetum. The behaviour of these wall layers was more or less normal and similar in both treated and control plants until the beginning of meiosis. The cells of the middle layer in the control plants degenerated at early vacuolated pollen grain stages, while in the anthers of treated plants, these cells persisted till maturity. The endothecial cells in control plants elongated tangentially, but with the commencement of tapetal degeneration, they elongated radially. On complete degeneration of the tapetum, cells in the endothecial layer attained maximum radial enlargement and characteristic fibrous thickenings appeared on their radial walls (Fig. 1). However, the endothecial cells in the treated plants, exhibiting 95-100% pollen sterility, continued to elongate in tangential direction even after the formation of pollen grains and the characteristic fibrous bands failed to appear on their radial walls (Fig. 5).

During pre-meiotic stages, the tapetal cells in the anthers of control plants consisted of thin walls connected with one another and with sporogenous cells by plasmodesmata. Plastids, mitochondria and a limited number of dictyosomes were present in these cells. At late pre-meiotic stages, ribosomes with smooth ER, mitochondria, plastids and a large number of pro-orbicular bodies were prominent. Degeneration of tapetal cells commenced at the microspore tetrad stage. At this stage, vacuoles appeared in tapetal protoplast and pro-orbicular bodies acquired a coat of electron dense material followed by deposition of sporopollenin on the orbicular wall, while the microspores were still enclosed in the common callose wall. Further disintegration of tapetal cells continued after liberation of microspores from the common callose wall and formation of vacuolate pollen grains. This was marked by increased vacuolation and progressive degeneration of nuclear envelope, ER, mitochondria, plastids and plasmalemma (Fig. 2). This was accompanied with the appearance of several large Ubisch bodies on the inner tangential tapetal wall (Fig. 3). Finally, the tapetal cells had been completely absorbed by the time pollen grains were engorged with reserves (Figs.1, 4).

On the other hand, the tapetal cells in the anthers of treated plants, exhibiting 100% pollen sterility, failed to show signs of degeneration and remained intact even after the formation of pollen grains (Fig.5). The tapetal cytoplasm was highly vacuolated at the microspore tetrad stage. Interestingly, a large

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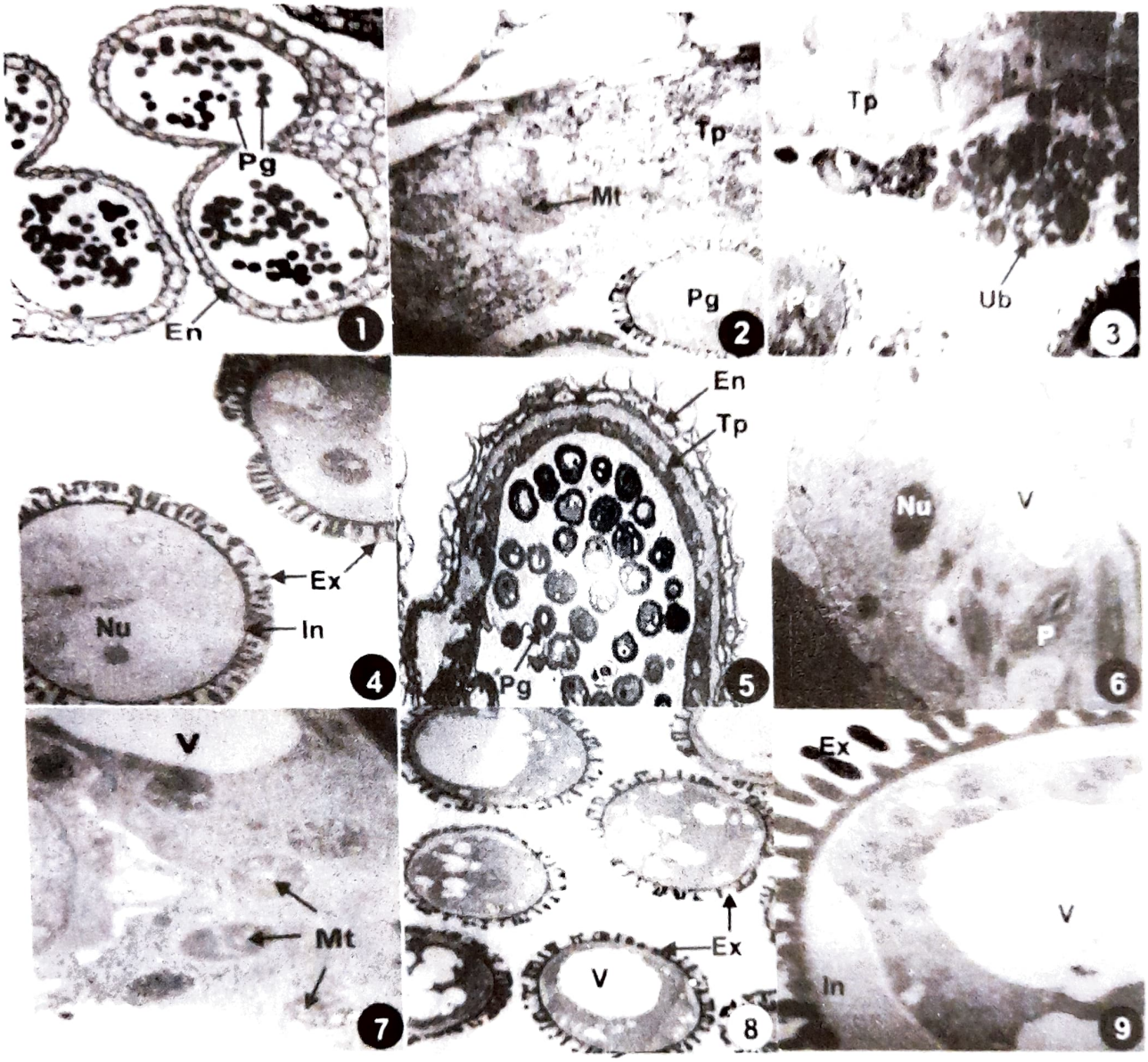

Figures 1-9: Light microscopic (LM) and Transmission electron microscopic (TEM) views of anthers of surf excel treated and untreated plants of *Raphanus sativus* L.

1: LM photograph of mature anther of untreated plant at mature pollen grain stage. Note the presence of fibrous bands in the endothecial cells (En). 230 X. 2: TEM image of part of anther wall of the control plant showing tapetum (Tp) at vacuolate pollen grain stage. Note the presence of degenerating mitochondria (Mt), plastids and large number of ribosomes. 1100 X. 3: TEM image of part of anther wall of untreated plants showing tapetal cells (Tp) at engorged pollen grains. Note the presence of large number of Ubisch bodies (Ub) on inner tangential surface. 1100 X. 4: Mature pollen grains engorged with reserves in the anthers of control plant. Note well-developed exine (Ex) with tactum and baculae, intine (In) and nucleus (Nu). 1500 X. 5: LM photograph of part of mature anther of treated plant at pollen grain stage. Note intact tapetum (Tp), highly vacuolated sterile pollen grains (Pg) and absence of fibrous bands in the endothecial cells (En). 530 X. 6: TEM image of tapetal cells of treated plant at pollen grain stage. Note the presence of large vacuoles (V), enlarged and degenerated nucleus (Nu) and plastids (P). 2400 X. 7: TEM image of part of tapetal cell of treated plant. Note highly vacuolated (V) cytoplasm, degenerated and thick-walled mitochondria (Mt). 2400 X. 8: Mature sterile pollen grains of treated plant. Note highly vacuolated (V) cytoplasm and degenerating nucleus (Nu) and absence of baculae.1100 X. 9: Part of sterile pollen grains of treated plants. Note the presence of large vacuole (V), exine with tectum alone and thick intine (In). 2100 X

number of small and thick-walled mitochondria were present in these tapetal cells. However, soon after the liberation of microspores from the common callose wall, these mitochondria degenerated. At this stage, all other organelles, particularly plastids, also exhibited signs of disintegration (Fig. 6). There was a reduction in the formation of pro-orbicules but sporopollenin synthesis was not much influenced. Enlargement of tapetal cells continued even after the formation of vacuolate pollen grains. These enlarged and intact tapetal cells consisted of large vacuoles with mitochondria, plastids and dictyosomes all in fully degenerated condition (Figs. 6, 7). Association of persistence of tapetum with pollen abortion has been reported in a large number of cytoplasmic, genic, gene-cytoplasmic and chemically induced male sterile plants (Chauhan and Kinoshita, 1982 and Kaul, 1988). Abnormal degeneration of tapetal mitochondria and alteration in its genome are well known in several cytoplasmic male sterile (CMS) plants (see Chauhan and Kinoshita, 1995; Raghvan, 1997 and Shivanna, 2003). Recently, increase in the number of tapetal mitochondria and their pre-mature degeneration have been observed in the anthers of surf-excel treated plants of *Brassica juncea* (Chauhan and Singh, 2006), *Cicer arietinum* (Chauhan and Gupta, 2006) and *Cyamopsis tetragonoloba* (Chauhan and Nisha, 2006). These authors hold that increase in the number of mitochondria in the tapetal cells reflects an increase in their metabolic activity. However, the sudden collapse of mitochondria results in abrupt reduction in the energy levels, and due to starvation, the tapetal cells enlarge in radial direction in search of nutrition, as enunciated by Chauhan and Singh (1968) in the case of genic male sterile *Cucumis melo*. The tapetal cells fail to degenerate, and thus fail to provide nutrition to the developing microspores, which finally become sterile.

The pollen mother cells (PMCs) in both fertile as well as sterile anthers underwent normal meiotic division, and both of the component divisions took place in quick succession to convert PMCs into tetrads of microspores, each of which is surrounded by a callose wall. On the release from the callose wall each microspore in the anthers of control plants developed into a pollen grain with a thick exine and thin intine. The pollen grains were spherical and tricolpate (Fig. 4). The sterile pollen grains in the anthers of treated plants were of various shapes and size. The protoplast of these pollen grains consisted of several small and a large central vacuoles (Fig. 8). Several small degenerated mitochondria were present in the vacuolated protoplast indicating their sterile state. However, formation of exine was not much affected, except that the formation of baculae was severely influenced (Fig. 9), thus indicating clearly that the synthesis of sporopollenin in intact tapetal cells was not much influenced. However, the intine at places was abnormally thick (Figs. 8, 9).

Thus, pollen abortion in the anthers of surf excel treated plants of *Raphanus sativus* is associated with the abnormal behaviour of tapetal mitochondria as is known for several CMS plants.

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