MORPHOLOGICAL AND HISTOCHEMICAL STUDIES ON *CAPSICUM ANNUUM* L. PLANTS INFECTED WITH CUCUMBER MOSAIC VIRUS

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PLANTS infected by viruses show symptoms on all the parts including their flowers[1]. The anthers and ovules of such flowers fail to develop normally and production of seeds and fruits is much inhibited. The reduction in pollen fertility in virus infected plants of *Capsicum annuum* has been recorded[2] and histopathological studies on their reproductive organs have also been made[3]. In order to find out the exact causes of male sterility in *Capsicum annuum* plants infected by cucumber mosaic virus, a comparative morphological and histochemical study was undertaken.

The seeds of *C. annuum* var. N. P. 46 were sown in the pots and plants thus raised were kept in the glass house. These plants were mechanically inoculated with the purified sap of infected chilli plants by the method after Yarwood[4] at four different stages of growth, *viz.*, (i) two weeks before floral bud initiation, (ii) one week prior to floral bud initiation, (iii) at the time of floral bud initiation and (iv) after blossoming (see Table I). The purification of cucumber mosaic virus was done by the procedure described by Steere[5]. Some plants were left to serve as control. The extent of pollen sterility in control as well as in inoculated plants was checked by the method of Alexander[6]. The flower buds of these plants were fixed in formalin-acetic-alcohol and these were dehydrated, cleared and embedded in paraffin by customary methods. The sections were cut at 7–12 μ and were stained with Heidenhan's iron-alum haematoxylin. For histochemical localization of total carbohydrates of insoluble polysaccharides (PAS reaction), total proteins (ninhydrin-schiff's test), histones (alkaline fast green test) and DNA (feulgen reaction) in the microtomed sections the procedures described by Jensen[7] were followed.

The plants inoculated at different stages of growth exhibited variable degree of pollen sterility associated with abnormalities in tapetal behaviour and dehiscent and non-dehiscent nature of anthers (Table 1). In the following paragraphs these abnormalities in each group are described separately.

TABLE I

Extent of pollen sterility, dehiscent and non-dehiscent nature of anthers and tapetal behaviour in the anthers of Capsicum annum L. *plants inoculated with cucumber mosaic virus at different stages of plant growth*

Stage of inoculation	Anther dehiscent/ indehiscent	Pollen sterility (%)	Tapetal behaviour
I. After blossoming	Dehiscent	0–15	Degeneration at early vacuolate pollen stage.
II. At the time of floral bud initiation	Dehiscent	16–50	Degeneration at late vacuolate pollen stage
III. One week prior to floral bud initiation	(i) Dehiscent	51–65	Degeneration at engorged pollen stage.
	(ii) Partially dehiscent	66–80	In dehiscent microsporangia degeneration at engorged pollen stage. In non-dehiscent anther lobes tapetal cells persist upto anthesis.
	(iii) Indehiscent	81–95	Tapetal cells remain intact upto anthesis.
IV. Two weeks before floral bud initiation	Indehiscent	96–100	(i) Tapetal degeneration in premeiotic stage. (ii) Tapetal hypertrophy in pre- and post-meiotic stages.
Control	Dehiscent	0–15	Degeneration at early vacuolate pollen stage.

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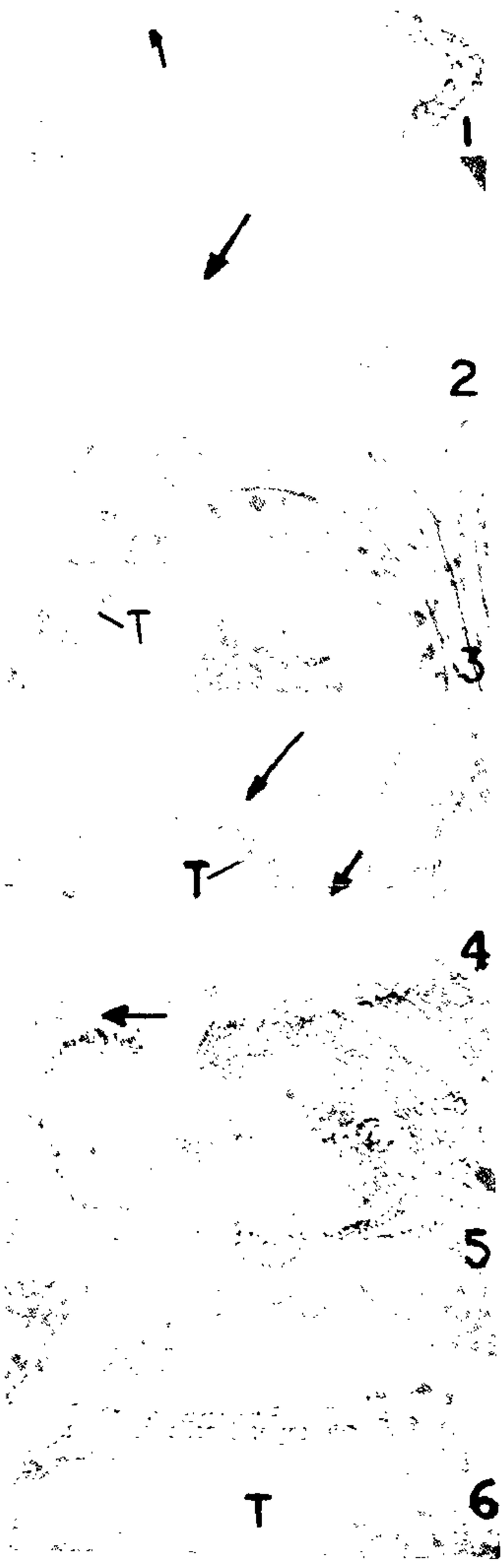


FIGS. 1–6. T.s. part of anther of *Capsicum annuum* plants inoculated at different stages of growth. Fig. 1. Pollen grains stage. Note the presence of degenerated tapetum, 100 ×. Fig. 2. Dehiscent anther at pollen grain stage showing degenerated tapetum, 280 ×. Fig. 3. Non-dehiscent anther at pollen grains stage showing intact tapetum (T), 280 ×. Fig. 4. Partially dehiscent anther at pollen grain stage showing degenerated tapetal protoplasts and fibrous bands in a few endothecial cells, 210 ×. Fig. 5. Tapetal degeneration at sporogenous tissue stage, 80 ×. Fig. 6. Tapetal hypertrophy at sporogenous tissue stage, 210 ×.

I. *Plants Inoculated after Blossoming*

The development of anthers in these plants was normal and resembled that of control plants. The tapetal cells degenerated at early vacuolate pollen stage. This was followed by the appearance of characteristic fibrous thickenings on the radial walls of the endothecial cells to facilitate normal anther dehiscence. The extent of pollen sterility was only 0–15% as was also exhibited by control plants.

II. *Plants inoculated at the time of floral bud initiation*

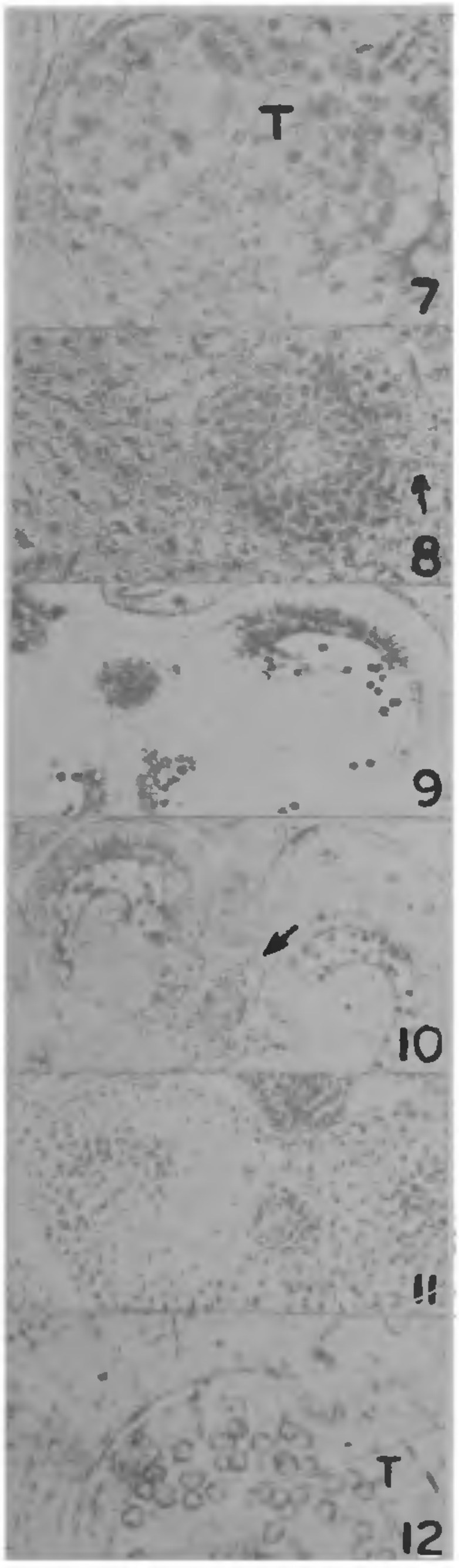
The development of anthers in such type of plants was more or less similar to those inoculated after flowering except that the tapetal degeneration was delayed to late vacuolate pollen stage (Fig. 1). The breakdown of tapetal cells was followed by the formation of fibrous bands in the endothecial cells to make the anthers dehiscent. Such anthers possessed 16–50% non-viable pollen grains.

III. *Plants inoculated one week prior to floral bud initiation*

The anthers of such plants were dehiscent, partially dehiscent or non-dehiscent. The development of dehiscent anthers of these plants resembled to that of plants inoculated at the time of floral bud initiation. However, tapetal degeneration was further delayed and the cells in this layer disintegrated only at engorged pollen stage (Fig. 2). Fibrous bands in endothecial cells appeared only after tapetal breakdown. The extent of pollen sterility in such anthers was 51–60%. On the other hand, the tapetal cells in non-dehiscent anthers failed to breakdown (Fig. 3). The intact tapetal cells possessed darkly stained degenerated nuclei. The endothecial cells in such anthers were devoid of fibrous bands to make them indehiscent. Pollen sterility in such anthers was 81–95%. In partially dehiscent anthers, fibrous bands appeared in the endothecial cells of 1–3 microsporangia (Fig. 4). It was interesting to note that in such anther lobes fibrous bands appeared only after tapetal degeneration. On the other hand, in the remaining microsporangia, the tapetal protoplast was intact and the formation of fibrous bands was inhibited. The extent of pollen sterility in such anthers was 61–80%. Development of endothecium in relation to tapetal behaviour in some cytoplasmic, genic as well as chemically induced male sterile plants has earlier been recorded.[8,9]

IV. *Plants inoculated two weeks before floral bud initiation*

The wall layers, tapetum in particular in the anthers of these plants exhibited abnormalities in both pre-



FIGS. 7-12. T.s. part of anthers of control and *Capsicum annuum* plants inoculated two weeks prior to floral bud initiation. Fig. 7. Tapetal (T) hypertrophy at pollen grain stage, 280 ×. Fig. 8. Anther connective showing vascular inhibition and hyaline granules, 280 ×. Fig. 9. Localization of histones at pollen stage in the anthers of control plants, 120 ×. Fig. 10. PAS reaction in the anthers of control plants at pollen stage, 120 ×. Fig. 11. Localization of protein in the anthers of complete sterile plants at sporogenous tissue stage, 120 ×. Fig. 12. Localization of DNA in the anthers of complete sterile plants at pollen grain stage, 210 ×.

and post-meiotic stages. The anthers were indehiscent with 96-100% non-viable pollen grains.

A. *Degeneration of tapetal cells in pre-meiotic stages* : In a limited number of anthers of these plants, the tapetal cells degenerated at sporogenous tissue stage (Fig. 5). This was followed by the breakdown of sporogenous cells. The vascular tissue in such anthers was procambial and the surrounding parenchyma cells showed the presence of hyaline granules.

B. *Hypertrophy of tapetal cells in pre- and post-meiotic stages* : In the anthers of large number of plants, the tapetal cells enlarged radially and became hypertrophied either during pre- or post-meiotic stages (Figs. 6, 7). The abnormally enlarged tapetal cells were highly vacuolated and possessed poorly stained scanty cytoplasm and degenerated nuclei. The hypertrophied tapetal cells either crushed the sporogenous cells or pollen mother cells much prior to the onset of meiosis or in post-meiotic stages crushed the microspores or the pollen grains. The cells in the endothecial layers elongated tangentially and fibrous bands on their radial walls failed to appear. The vascular strand either remained pro-cambial or only a limited number of thin-walled xylem elements appeared with surrounding parenchyma showing signs of degeneration (Fig. 8). These cells possessed hyaline granules.

These observations have clearly demonstrated that vascular inhibition in the anthers of infected chilli plants resulted into tapetal abnormalities causing pollen sterility of various degrees. Partial or complete pollen sterility caused by tapetal abnormalities due to vascular inhibition in cytoplasmic as well as in chemically induced male sterile plants of *Capsicum annuum* has earlier been recorded[11-12]. Another notable finding of the present study was the presence of hyaline granules in the anther connective of plants exhibiting complete pollen sterility. Similar observations have also been made in some cytoplasmic and chemically induced male sterile plants including pepper[13,14]. The formation of hyaline granules in the anther connective of sterile plants may be the result of depletion of vascular supply caused by internal factors[14].

Histochemical localization of total carbohydrates of insoluble polysaccharides (PAS test), total proteins (ninhydrin-schiff's test), histones (alkaline-fast green test) and DNA (Feulgen reaction) in the anthers of

control plants indicated that the intensity of various reactions gradually increased and the engorged pollen grains showed the highest concentrations of these substances (Figs. 9, 10). On the other hand, in the anthers of plants inoculated at different stages of growth, the concentration of these substances decreased with the increase in the extent of pollen sterility. The plants inoculated a fortnight prior to the floral bud initiation exhibiting complete pollen sterility showed inconspicuous PAS reaction and lower amounts of proteins, histones and DNA in different parts of anther including the malformed tapetum and pollen grains (Figs. 11, 12). Similar findings in some cytoplasmic, genic and chemically induced male sterile plants have also been recorded[15].

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BACILLUS CEREUS AS A CAUSE OF ABORTION IN A MARE

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Bacillus cereus, first described by Frankland and Frankland in the year 1887, is generally considered as a saprophyte[1]. Occasionally, this organism has been incriminated in food poisoning in man[2] and in casts of abortions in cattle[3]. However, there are no records of the involvement of this organism in equine abortions. This report documents the isolation of *B. cereus* from an aborted equine foetus and the cervical mucus of the aborted mare.

During the study of the bacterial flora of the cervical mucus of mares and aborted equine foetuses, *B. cereus* was isolated from an aborted foetus and its mare. The aborted mare, a thoroughbred, was barren during the last two seasons prior to the abortive conception. The mare aborted around 160 days post-conception. The foetus did not show any gross abnormalities. The heart blood and the stomach contents of the foetus and the cervical mucus of the mare were collected aseptically and cultured on 8% blood agar plates. Incubation was carried out at 37° C under 10% carbon-dioxide tension for 48 hr before examination.

Examination of the plates inoculated with the heart blood, stomach contents and cervical mucus revealed the presence of similar colonies. They were 1–2 mm in diameter, greyish-white with a rough surface. The margins were undulating. The colonies were surrounded by a zone of beta haemolysis. Microscopical examination after gram staining of the smears of the colonies from the three plates showed the organisms as gram positive sporulating rods. The spores were located centrally. The organisms were identified as *Bacillus cereus*[2].

Although, *B. cereus* is generally considered as a saprophyte, its saprophytic nature should not be viewed lightly as it has been incriminated as a primary pathogen in three cases of bovine abortions in three different herds[3]. In the present report, the isolation of *B. cereus* from the heart blood and stomach contents of the foetus and the cervical mucus of the aborted mare points to the possibility of a positive role played by this organism in equine abortion. Available literature does not reveal the involvement of *B. cereus* in equine abortion. This report therefore is the first of its kind.

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