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HISTOLOGICAL AND HISTOCHEMICAL CHANGES IN THE ANTHERS OF SOME DISEASED VEGETABLE CROPS

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Introduction

Since biblical times there has been speculation on the causes, care, cure and prevention of diseases. Scientists have done a great deal in the measurements of crop losses due to diseases, but not much is known about how continuous deformation of diseased plants results on reducing crop yield. According to ROBERTS and BOOTHROYD[16], the most important contribution in the near future will come from studies designed and oriented to learn the extent of plant diseases rendering no significant loss that we can afford. In order to understand the exact causes of reduction in yield, histological and histochemical changes in the anthers of variously infected vegetable crops were studied.

Materials and Methods

The seeds of *Capsicum annuum* L. var. N. P. 46, *Coriandrum sativum* L. var. Bichpuri local, *Raphanus sativus* L. var. Pusa desi and *Solanum melongena* L. var. T_2 were sown in pots as well as in the fields of the Botanical gardens, R. B. S. College, Agra.

The plants thus raised were inoculated at either (a) pre-floral bud initiation stage or (b) after floral bud initiation stage or (c) at the time of anthesis by following procedures:

1) *Capsicum annuum* plants were mechanically inoculated after method of YARWOOD (1957) with the sap containing cucumber mosaic virus.

2) *Coriandrum sativum* plants were inoculated by addition of suspension of chlamydospores of the fungus *Protomyces macrosporous* Ung. The suspension was obtained by crushing the hypertrophied plant parts of the host in distilled water.

3) *Raphanus sativus* plants grown in glass houses were inoculated with the nymphs of the aphid *Lipaphis erysimi* Kalt.

4) *Solanum melongena* plants grown in 30 cm earthern pots containing 2.5 kg of steam-sterilized sandy loam soil were inoculated with second stage larvae of *Meloidogyne incognita* Kojoid and White.

The pollen viability of inoculated and control plants was tested after the method of ALEXANDER[2]. The floral buds of these plants were fixed in formalin-acetic-alcohol for histological studies and for various histochemical studies, the buds were fixed in 1 : 3 acetic-alcohol, 80% acetone, 10% formalin and 80% alcohol. These floral buds were dehydrated, cleared and embedded by usual method. The sections were cut at 7～16 μ and were stained with HEIDENHAIN's iron-alum haemotoxylin for histological studies. For histochemical localization of total carbohydrates of insoluble polysaccharides, total proteins, histones, DNA and enzyme acid phosphatase, the procedure described by JENSEN[12] were followed.

Observations

1. Histological

The infected plants, especially those inoculated at pre-floral bud initiation stage exhibit a great reduction in the number of flowers and fruits and their pollen viability is also much influenced. Based on the extent of pollen sterility caused, the infected plants are grouped in three classes namely: i) normal (N), ii) semi-sterile (S. S.) and iii) complete sterile (C. S.).

Early anther ontogeny in the above mentioned groups of inoculated plants is more or less similar to those of control. However, after the differentiation of various wall layers in an anther, infected plants exhibit abnormalities.

i) Normal: The plants inoculated at the time of anthesis exhibit normal anther development and possess only 0～15% non-viable pollen grains (Fig. 1).

ii) S. S.: The plants inoculated at the time of floral bud initiation belong to this class. The anthers of these plants are partially sterile and exhibit great variation in the extent of pollen sterility and are either dehiscent or non-dehiscent in the same flower at different rates of mixing.

The dehiscent anthers possess 16～55% non-vaible pollen grains and

their development is more or less similar to N type of plants except that the degeneration of tapetal cells is delayed and formation of characteristic fibrous thicknings on the radial walls of endothecium occurs only after complete degeneration of tapetal cells (Fig. 2). On the other hand, indehiscent anthers contain 56~95% sterile pollen grains and tapetal cells in such anthers persist up to anthesis (Fig. 3). The fibrous bands in the endothecial cells of such anthers fail to appear. The vascular strand in the anther connective is also poorly differentiated and consists of a limited number of thin walled, narrow xylem elements enclosed by phloem tissue showing signs of degeneration.

iii) C. S.: The plants inoculated at pre-floral bud initiation stage are of this class. The anthers of this group of plants are completely indehiscent and contain 96~100% non-viable pollen grains. These anthers exhibit abnormalities in their wall layers, sporogenous cells and pollen grains in both pre-and post-meiotic stages.

a. *Abnormalities in pre-meiotic stages*: The tapetal cells either degenerate (Fig. 4) or become abnormally enlarged (Fig. 5) much prior to the onset of meiosis. In the former condition, the sporogeneous cells degenerate and in the latter case, the sporogenous cells get crushed mechanically by radially elongating tapetal cells. The vascular strand in such anthers remains pro-cambial.

b. *Abnormalities in post-meiotic stages*: The tapetal cells fail to degenerate and not only remain intact but elongate radially and become hypertrophied either at microspore tetrad stage (Fig. 6), or at microspore stage or at vacuolate pollen grain stage (Fig. 7). In either case, the hypertrophied tapetal cells occlude the anther sac and crush its components. The hypertrophied tapetal cells are highly vacuolated and possess scanty and feebly stained cytoplasm and degenerated nuclei. In a limited number of anthers of *R. sativus* plants of this class, the protoplasmic contents of the tapetal cells invade the anther sac in the form of a pseudoperiplasmodium (Fig. 8). The endothecial cells in the anthers of C. S. type of plants fail to produce fibrous bands on their radial walls. The vascular differentiation in such anthers is also inhibited (Figs. 4, 6, 7 & 8).

2. Histochemical

Various histochemical tests made to localize total carbohydrates of insoluble polysaccharides, total proteins, histones, DNA and enzyme acid phosphatase in various parts of an anther at different stages of development indicate that with the increase in pollen sterility in variously inoculated plants, the intensity of these reactions decline. The various wall layers of an anther

tapetum in particular and pollen grains of the plants inoculated at pre-floral bud initiation stage (C. S. type) exhibit poor histochemical reaction indicating marked deficiency of these substances (Fig. 9). Poor enzyme acid phosphatase activity in the malformed tapeta of various types in such anthers reflects on their low metabolic state (Fig. 10).

Discussion

The foregoing description on histological changes in the anthers of variously inoculated plants exhibit pollen sterility of various degree. The plants inoculated at pre-floral bud initiation stage become completely sterile. Earlier, OHTA[15] had also observed the reduction in pollen sterility in virus infected plants of *Capsicum annuum.* The anthers of infected plants exhibit abnormalities in endothecium, tapetum and vascular strand. The formation of fibrous bands on the radial walls of endothecial cells is inhibited. This is associated with the malformed tapeta of various types. These findings support the hypothesis of DEFOSSARD[9] on endothecium development in *in vitro* grown plants of *Chenopodium rubrum.* According to him, the formation of fibrous bands of endothecium occurs only after complete breakdown of tapetal cells. Similar observations in several male sterile plants have been recorded by CHAUHAN[5]. The abnormalities in tapetal behaviour observed are similar to those recorded in a large number of cytoplasmic, genic as well as chemically induced male sterile plants[7,10,13]. Anther development in *Coriandrum sativum* plants infected by *Protomyces macrosporous* and *Capsicum annuum* plants infected by cucumber mosaic virus has also been observed earlier by GUPTA[11] and AWASTHY and SINGH[3] respectively. However, these authors are silent about the behaviour of endothecium and tapetum. The vascular differentiation in the anthers of inoculated plants exhibiting complete sterility is also much inhibited. This in the opinion of the present authors, prevents various nutrients reaching the developing pollen grains *via* tapetum. Consequently, tapetal layer exhibit abnormalities of various types and pollen grains degenerate. It is strongly corroborated by the present histochemical observations indicating the deficiency of total carbohydrates, total proteins, histones and DNA in various parts of an anther including tapetum. Poor enzyme acid phosphatase activity in malformed tapetal cells indicate their low metabloic state. Similar findings have been recorded in a large number of cytoplasmic, genic as well as chemically induced male sterile plants[1,4,6,8,14].

Summary

Hisotological and histochemical changes in the anthers of *Capsicum annuum* plants infected by cucumber mosaic virus, *Coriandrum sativum* plants infected by the fungus *Protomyces macrosporous, Raphanus sativus* plants infected by an aphid *Lipaphis erysimi* and *Solanum melongena* plants infected by a nematode *Meloidogyne incognita* were studied. Plants inoculated with their respective parasites at different stages of growth exhibited pollen sterility of variable degree and those inoculated at pre-floral bud initiation stage were complete male sterile. The anthers of such plants exhibited abnormalities in the behaviour of endothecium, tapetum and vascular strand. It is concluded on the basis of these findings that development of endothecium is controlled by tapetum and malfunctioning tapeta of various types is the result of vascular inhibition. This is strongly corroborated by the present histochemical findings indicating deficiency of total carbohydrates of insoluble polysaccharides, total proteins, histones and DNA. The low metabolic state of malformed tapeta was also reflected by their poor enzyme acid phosphatase activity. It is further concluded that the effect of various pathogens in causing pollen sterility is similar to plants where male sterility is either genetically controlled or induced by gametocides.

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Legend for Plate I

Figs. 1–10. Transverse part of anthers of variously infected plants.

Fig. 1. *R. sativus.* N type at vacuolate pollen stage. 100×

Fig. 2. *C. sativum.* S. S. type at pollen grain stage. Note the presence of chlamydospores (CH) in the connective and fibrous bands in endothecium (E). 120×

Fig. 3. *C. annuum.* S. S. type at vacuolate pollen stage.

Fig. 4. *S. melongena.* C. S. type showing degeneration of tapetum at sporogenous tissue (ST) stage. 80×

Fig. 5. *C. sativum.* C. S. type. Hypertrophy of tapetal cells at sporogenous tissue stage. 80×

Fig. 6. *R. sativus.* C. S. type. Hypertrophy of tapetum at microspore tetrad (MT) stage. 110×

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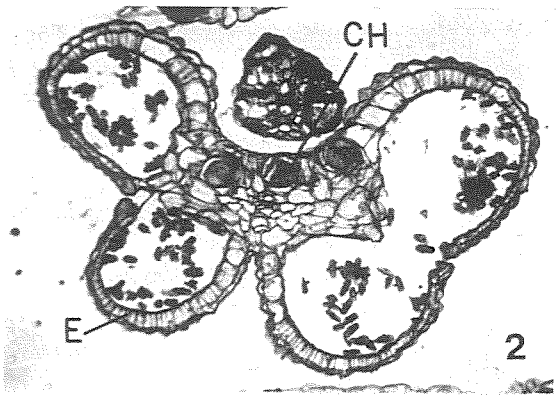
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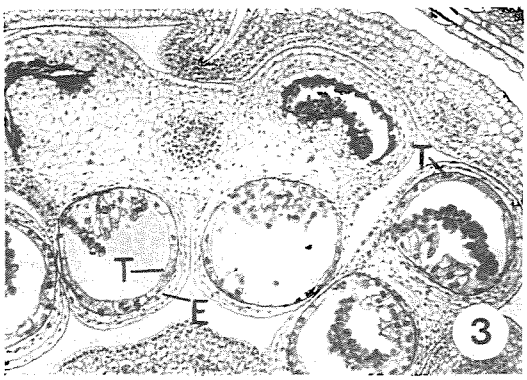
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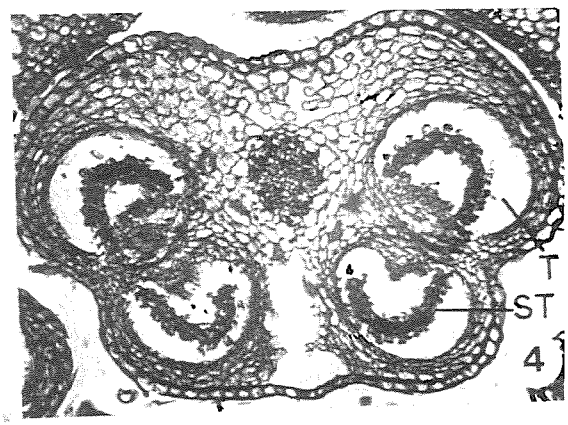
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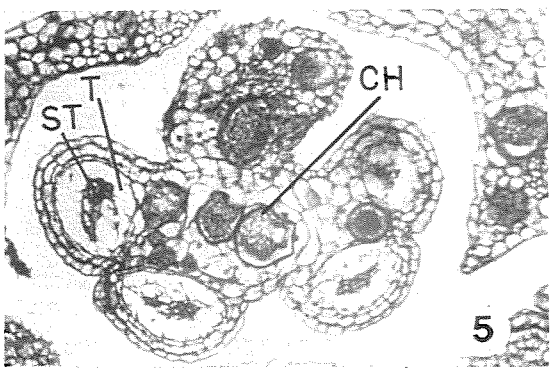
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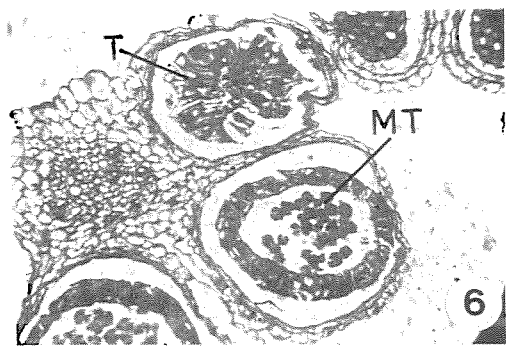
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Legend for Plate II

Fig. 7. *C. annuum*. C. S. type. Hypertrophy of tapetal cells at pollen grain stage. 210×

Fig. 8. *R. sativus*. C. S. type. Formation of pseudoperiplasmodium (PL) at micropore tetrad stage. 110×

Fig. 9. *S. melongena*. C. S. type showing poor PAS reaction indicating deficiency of carbohydrates at pollen (P) stage. 210×

Fig. 10. *R. sativus*. C. S. type. Poor enzyme acid phosphatase activity in tapetum and pollen grains. 210×

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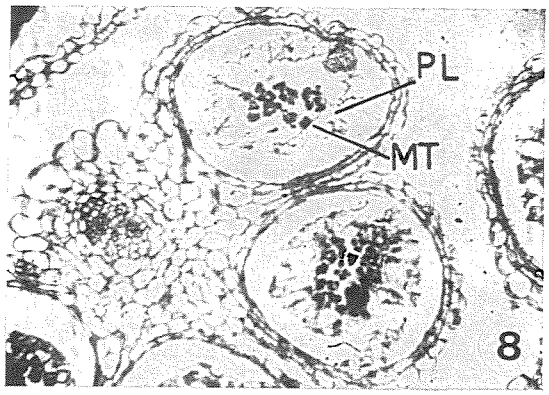
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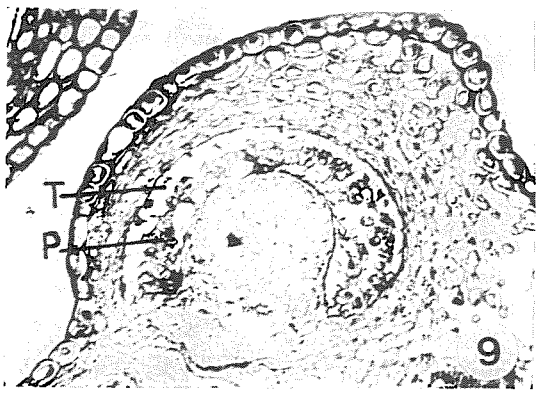
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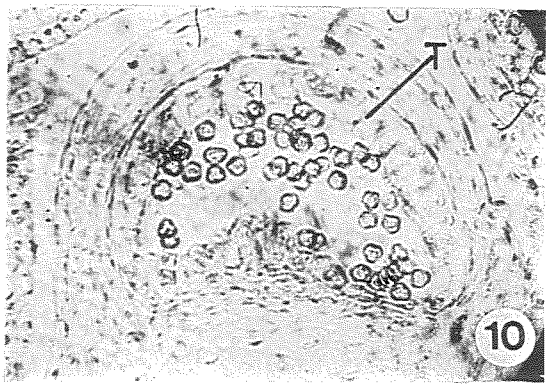
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