HISTOLOGICAL, HISTOCHEMICAL AND BIOCHEMICAL CHANGES IN THE ANTHERS OF *SOLANUM NIGRUM* L. PLANTS INFESTED BY *APHIS SPIRALCOLA* PATCH.

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Introduction

Substantial literature has appeared on histopathological and biochemical changes induced in the vegetative plant parts infested by aphids[1,6,17]. However, the manifestation of aphids on floral parts has received less attention[18,19]. The present investigation was undertaken with the aim of finding histopathological and biochemical changes in the anthers of *Solanum nigrum* plants infested by a sap sucking insect *Aphis spiraecola*.

Material and Methods

The seeds of *S. nigrum* collected from the fields of Agra district were sown in earthern pots. The plants thus raised were inoculated with the nymphs of the aphid *Aphis spiraecola* patch (Order : Hemiptera ; family ; Aphidide) a fortnight before floral bud initiation. Some plants were left to serve as controls. The pollen sterility of these plants was checked by the procedure after ALEXANDER[3]. For histopathological and histochemical studies, the floral buds of infested and control plants were fixed in formalin-acetic-alcohol. These were processes by customary schedules and sections were cut at 6–12 μm. For histopathological studies, these sections were stained with Heidenhain's iron-alum haematoxylin. For histochemical localization of total carbohydrates of insoluble polysaccharides in the microtomed sections, periodic Schiff's (PAS) test as described by JENSEN[14] was followed.

Total proteins in the anthers of infested and control plants at the different stages of anther development were estimated quantitatively by LOWRY *et al.*[16] in weighed dry and ground samples using a standard curve prepared from Bovine serum albumin.

Free proline in the anthers of infested and control plants at different stages of anther developmant was quantitatively estimated by the colorimeteric method of BATES *et al.*[4]. Standard curve was made by using pure proline (B. D. H.).

Experimental Results

I. HISTOPATHOLOGICAL:

A. *Pollen Sterility*: The infested plants exhibited variable degrees of pollen sterility. The extent of sterility was directly proportional to the intensity of infestation, markedly influenced by humidity and shade (Figs. 1, 2). Based on the extent of pollen sterility caused by the aphid infestation, the aphid infested and control plants were grouped into four classes, namely; (i) Control (*C*) plants with dehiscent anthers containing 0-20% non-viable pollen grains, (ii) plants infested by 5-15 insects per plant were more or less normal (*N*). The anthers of such plants were dehiscent with 0-20% sterile pollen, (iii) semi-sterile (*S. S.*) type of plants infested by 16-100 aphids per plant. Such plants exhibited most the variable degrees of pollen sterility ranging between 21-95%. The anthers of these plants were dehiscent, partially dehiscent or non-dehiscent and (iv) Complete sterile (*C. S.*) plants infested severely by more than 100 aphids per plants. The anthers of these plants were usually indehiscent and possessed 96-100% sterile pollen grains.

B. *Anther Development*: Control and *N* type infested plants exhibited normal development. However, anther wall layers and connective parenchyma in *S. S.* and *C. S.* type of infested plants exhibited abnormalities. In the following paragraphs, the description is limited to these groups where they exhibited deviations from normal course.

a. *S. S. Type*: The behaviour of various anther wall layers, especially that of endothecium and tapetum and anther connective was quite variable. The abmormalities exhibited by various parts of an anther were directly proportional to the extent of pollen sterility. The anthers with pollen sterility ranging between 21-50% exhibited more or less normal development (Fig. 3). However, the degeneration of tapetum was delayed. The cells in the endothecium became very tangentially streched and formation of characteristic fibrous bands on their radial walls was completely inhibited to make the anthers indehiscent (Fig. 4). The anthers exhibiting 51-65% pollen sterility

were partially dehiscent (Fig. 5). In such anthers, the microsporangia situated towards the center were dehiscent, while the microsporangia on the corolla side were indehiscent. The behaviour of tapetum in the dehiscent microsporangia resembled that of completely dehiscent anthers of this group of plants, while the degeneration of tapetum in the indehiscent sporangia was further delayed and only when the viable pollen grains were engorged with reserves, was the tapetal breakdown complete (Fig. 6).

The completely non-dehiscent anthers exhibited high degree of pollen sterility (66–95%) among this group of plants. The degeneration of tapetum was further delayed. The formation of fibrous thickenings on the radial walls of endothecial cells in such anthers was completely inhibited (Figs. 7, 8).

b. *C. S. Type*: Anthers of this group of plants exhibited various abnormalitiles in both pre- and post-meiotic stages. The anther lobes in severely infested floral buds at early sporogenous tissue along with the tapetum in such anthers degenerated much prior to the onset of meiosis (Fig. 8). On the other hand, in most of the anthers, the tapetal cells exhibited radial enlargement at meiosis I and II stage (Fig. 10). The abnormal enlargement of tapetal cells continued up to microspore tetrad stage (Figs. 11, 12). Beyond this stage, the tapetal cells showed tangential elongation and their protoplasts remained intact up to anthesis (Figs. 13, 14). The endotheical cells in such anthers elongated radially but formation of fibrous bands on their radial walls was fully inhibited (Fig. 13). Resorption tissue also failed to function and made the anthers indehiscent (Fig. 14). The vascular strand in the anthers of this group of plants remained procambial through out and parenchyma cells in the connective region showed signs of degeneration and possessed small hyaline granules (Figs. 15, 16).

II. HISTOCHEMICAL:

A. *Localization of total carbohydrates of insoluble polysaccharides (PAS Test) in the anthers of control and infested plants*:

The evaluation of PAS reaction in the anthers of various groups of infested as well as control plants at various stages of anther development is given in Table 1. The intensity of the reaction is arbitrarily divided into four parts, viz, low or slight (+), moderate (++), intense or high (+++) and most intense or highest (++++).

As is evident from Table 1, the PAS reaction in various parts of an anther in control (*C*) and normal (*N*) plants at all stages was more or less similar. In the early stages, the reaction was slight in all the parts except the outer tangential walls of epidermal cells showing moderate reaction. The

TABLE 1. Evaluation of PAS reaction in the anthers of control and infested plants at different stages of development. The reaction is divided arbitrarily into four parts viz. slight or low (+), moderate (++), intense or high (+++) and highest or most intense (++++)

Stage of Development	Type of plant	Cuticle	Epid-ermis	Endo-thecium	Middle layers	Tape-tum	Sporo-genous tissue	Pollen mother cells	Micro-spore tetrads	Micro-spores	Pollen grains	Anther connec-tive
Premeiotic	*C & N*		++	+	+	+	+					+
	S. S.		+	+	+	+	+					++
	C. S.		+	+	+	+	+					+
Meiosis I & II	*C & N*		+++	++	+	++		++				++
	S. S.		++	+	+	+		++				+
	C. S.		++	+	+	+		+				+
Microspore Tetrad	*C & N*	+++	+++	++	+	+++		++				++
	S. S.		++	+	+	+			+			+
	C. S.		++	+	+	+			+			+
Microspore	*C & N*	+++	+++	+++		++				+++		+++
	S. S.	++	++	+		+				+		+
	C. S.		++	+		+				+		+
Pollen	*C & N*	+++	+++	+++		++					+++	+++
	S. S.		++	++	+	+					++	++
	C. S.		++	+	+						+	+

intensity of the reaction increased with age and reached its maximum at microspore tetrad stage. The microspores, soon after their release from the callose wall, showed a steady increase of the reaction and pollen grains, ready to shed, possessed a large amount of total carbohydrates of insoluble polysaccharides (TCIP). The anther wall layers and connective parenchyma cells showed the presence of TICP grain (Figs. 17, 18).

On the other hand, the intensity of PAS reaction in various parts of an anther of *S. S.* and *C. S.* type of infested plants failed to accelerate and was far below as compared to that of control plants (Figs. 19, 20). The connective parenchyma cells and anther wall layers either possess a limited number of TICP grains (Fig. 19) or these grains are completely absent (Fig. 20).

III. BIOCHEMICAL:

A. *Quantitative estimation of total proteins and free proline in the anthers of control and infested plants*:

The quantitative estimation of total proteins and free proline in the anthers of control and infested plants at different stages of development is given in Table 2.

TABLE 2. Quantitative estimation of total proteins and free proline in the anthers of control and *C. S.* type of infested plants at different stages of development

Stages of development	Total proteins (% dry wt.)		Free proline (μg/mg fresh wt.)	
	Control	*C. S.*	Control	*C. S.*
a. Pre-meiotic	2.5	9.5	Trace	Trace
b. Meiosis I & II, Tetrad and Microspore	3.4	1.2	0.097	Trace
c. Pollen grain prior to anther dehiscence	5.2	1.6	0.34	Trace

As is evident from Table 2, the quantity of proteins in the anthers of control plants increased considerably with age and anthers about to dehisce exhibited highest protein concentration. On the other hand, the anthers of *C. S.* type of infested plants were markedly deficient in proteins at all stages of development. Similarly, the amount of free proline in the anthers of control plants increase gradually with age and maximum was recorded at mature pollen grain stage, while only traces of free proline were found in the mature anthers of *C. S.* type of infested plants.

Discussion

From the foregoing description it is evident that *Solanum nigrum* plants infested by *Aphis spiraecola* exhibited pollen sterility of variable degrees. The extent of pollen sterility was directly proportional to the degree of infestation. Anther ontogeny exhibited that pollen abortion in infested plants was associated with abnormalities in tapetal behaviour. Similar observations in *Raphanus sativus* plants infested by an aphid *Lipaphis erysimi* KALT. have also been recorded earlier[20]. Malfunctioning of tapetum in infested plants was similar to that observed in a large number of cytoplasmic, genic and chemically induced male sterile plants. In the opinion of the present authors, the tapetal abnormalities, in all probabilities, resulted due to starvation caused by vascular inhibition in the anther connective. This is also corroborated by the present histochemical and biochemical findings showing marked deficiency of total carbohydrates of insoluble polysaccharides, and total proteins in the anthers of infested plants exhibiting a high degree of pollen sterility. Deficiency of total carbohydrates of insoluble polysaccharides, proteins, histones and nucleic acids in the anthers of several cytoplasmic, genic as well as chemically induced male sterile plants have also been reported[8,10]. Thus the effect of the aphid infestation on anther development and microsporogenesis is similar to that of cytoplasmic, genic factors and gametocidal compounds.

The anthers of heavily infested plants also exhibited marked deficiency of free proline. Proline is one of the major amino acids present in the pollen grains of most plants and is connected with the pollen fertility and sexual process in plants.

Another notable finding of the present study is the confirmation of DE FOSSARD's, concept of programmed control of tapetum on endothecial development. According to DE FOSSARD,[12] the development of endothecium is controlled by tapetum during major course of anther development and only after tapetal breakdown, the characteristic fibrous thickenings appear on the radial walls of endotheical cells. In the presently studied material, endotheical thickenings developed only after the tapetal breakdown in the anthers of infested plants irrespective of the degree of pollen sterility induced.

Summary

Solanum nigrum plants infested by *Aphis spiraecola* exhibited various degrees of pollen sterillity associated with malfunctioning of tapetum caused by vascular inhibition. Impairment in the tapetal developmant is also re-

flected by inhibition of endothecium development. Histochemical and biochemical findings showed a marked deficiency of total carbohydrates of insoluble polysaccharides, total proteins and free proline in the anthers of heavily infested plants.

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Figs. 1–2. *Solanum nigrum* plants infested by *Aphis spiraecola.*

Fig. 1. Infested plant. Note the presence of aphids on stem, leaves, flowers and fruits.

Fig. 2. Magnified view of the infested plant shown in Fig. 1.

Figs. 3–10. Transverse anthers of *S. S.* and *C. S.* type of infested plants.

Fig. 3. *S. S.* anthers with lower range of pollen sterility. 120×.

Fig. 4. Magnified view of the anther shown in Fig. 3. 310×.

Fig. 5. *S. S.* partially dehiscent anthers. 120×.

Fig. 6. Magnified view of the anther shown in Fig. 5. 410×.

Fig. 7. *S. S.* non-dehiscent anthers. 120×.

Fig. 8. Magnified view of the anther shown in Fig. 7. 410×.


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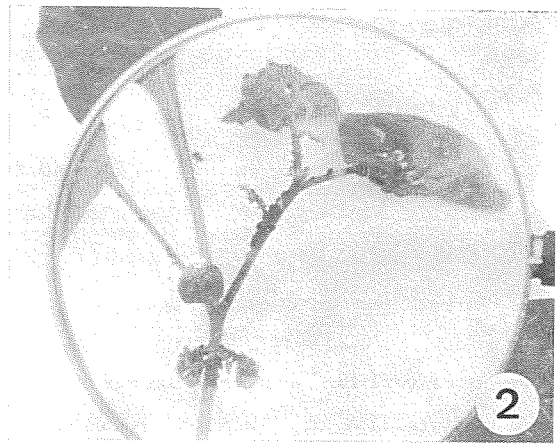
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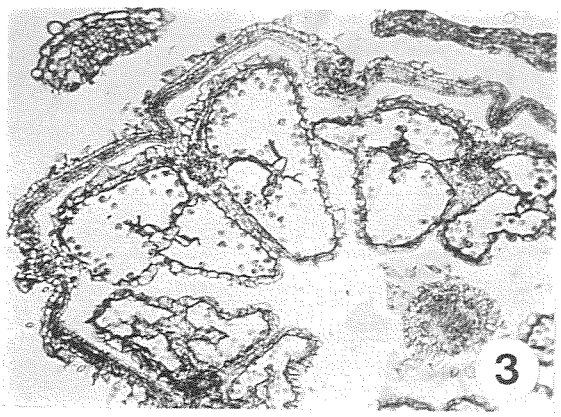
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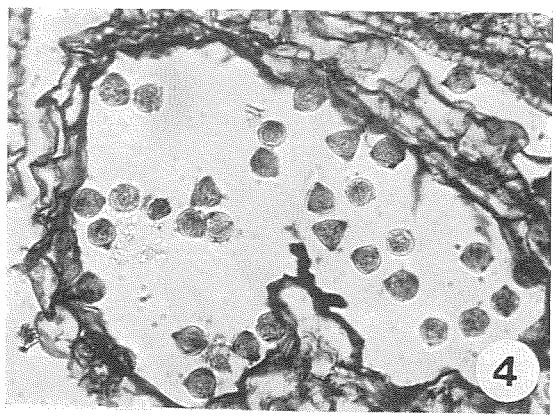
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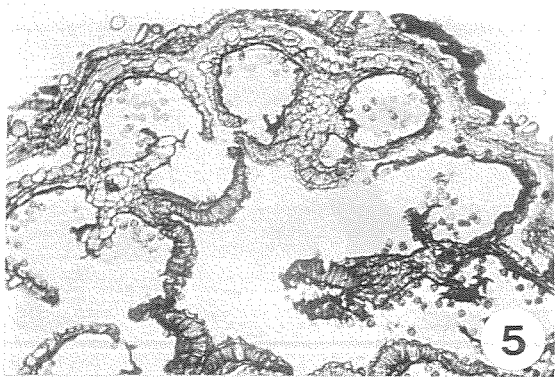
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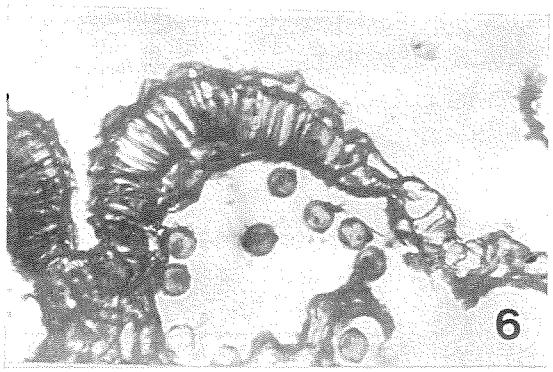
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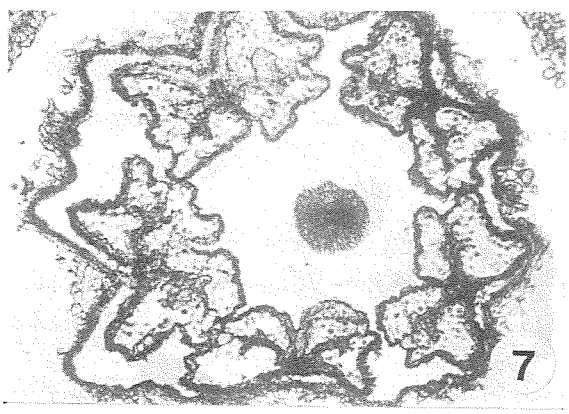
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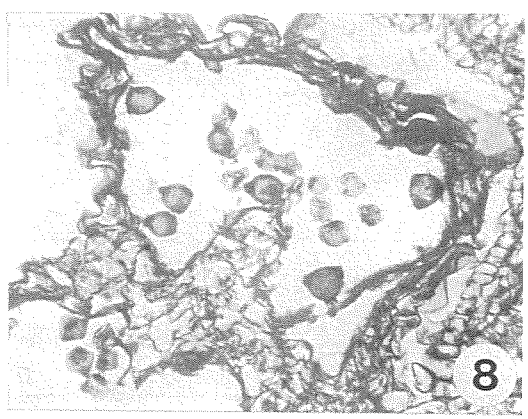
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Fig. 9. *C. S.* Anthers showing degeneration of sporogeneous tissue and tapetal cells. 120×.

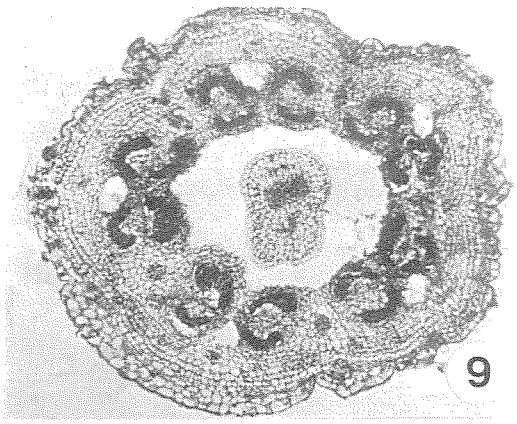
Fig. 10. *C. S.* Anthers showing tapetal enlargement in radial direction at pollen mother cell stage. 380×.

Fig. 11–16. Transverse anthers of *C. S.* type of infested plants.

Fig. 11 & 12. Microspore tetrad stage showing tapetal enlargement. 380×.

Fig. 13 & 14. Pollen grain stage showing intact tapetal protoplast. Note the absence of fibrous bands in endothecial cells and non-functional resoption tissue (RT). 380×.

Fig. 15 & 16. Pollen grain stage showing poorly developed vascular tissue and presence of hyaline granules in the connective parenchyma. 410×.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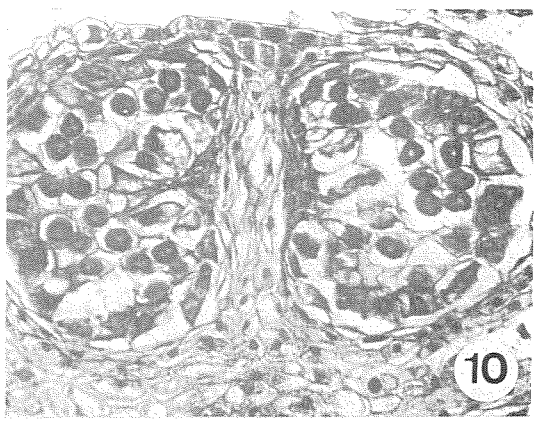
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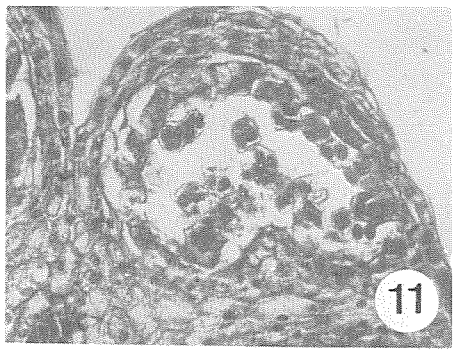
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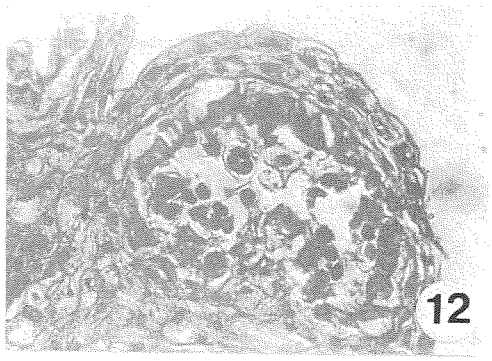
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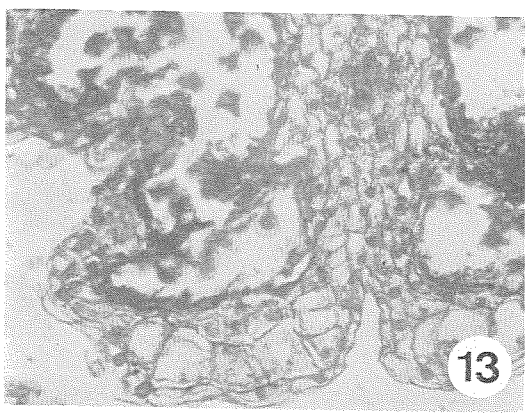
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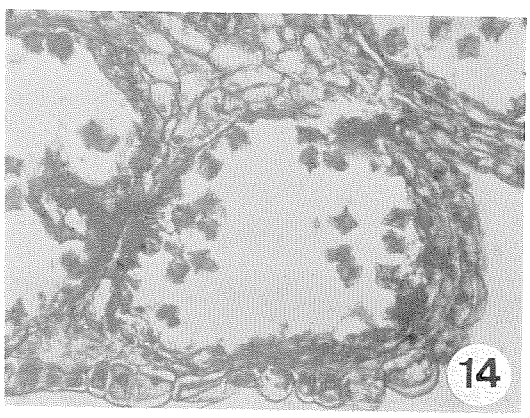
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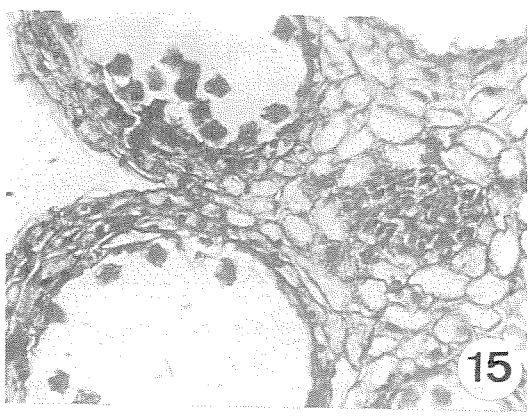
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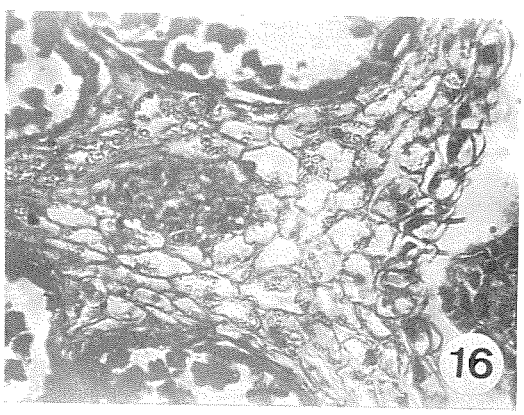
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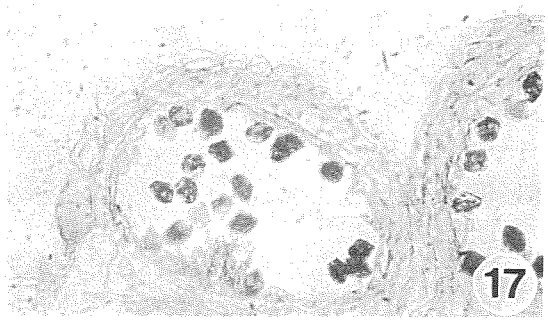
Figs. 17–20. Transverse anthers of *N*, *S. S.* and *C. S.* type of plants showing PAS reaction. 410×.

Fig. 17. *N* pollen grains with TCIP (total corbohydrates of insoluble polysaccharides) grains.

Fig. 18. *N* anther connective and wall layers showing the presence of TCIP grains.

Fig. 19. *S. S.* anther showing poor PAS reaction and reduction in the TCIP grains.

Fig. 20. *C. S.* anther showing poor PAS reaction and complete absence of TCIP grains.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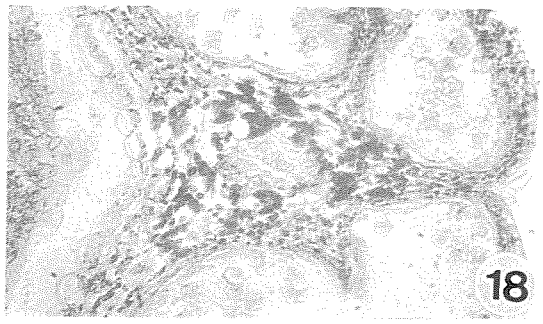
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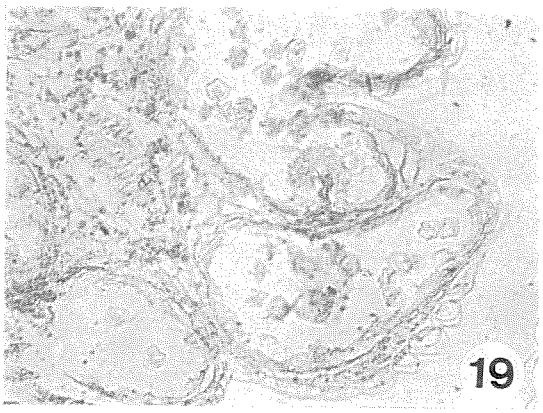
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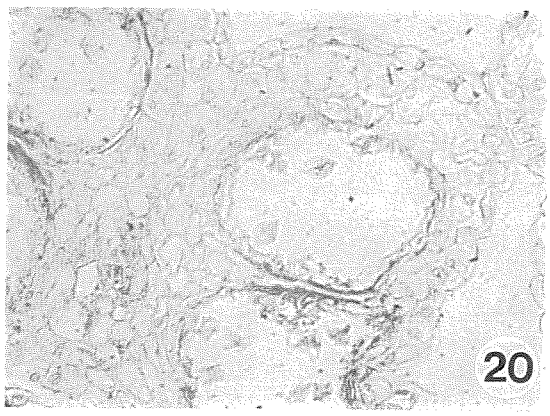
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