HOKKAIDO UNIVERSITY

| | |
|---|---|
| Title | Histochemical Studies in Some Cytoplasmic Male Sterile Lines of Wheat |
| Author(s) | Jain, A.K.; Rathore, R.K.S.; Chauhan, S.V.S. et al. |
| Citation | Journal of the Faculty of Agriculture, Hokkaido University, 61(1), 143-149 |
| Issue Date | 1982-11 |
| Doc URL | https://hdl.handle.net/2115/12975 |
| Type | departmental bulletin paper |
| File Information | 61(1)_p143-149.pdf |

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

# HISTOCHEMICAL STUDIES IN SOME CYTOPLASMIC MALE STERILE LINES OF WHEAT

A. K. JAIN*, R. K. S. RATHORE*, 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, Japan

Received April 28, 1982

## Introduction

The intimate relationship between developing pollen grains and tapetum has been shown by the work on cytoplasmic male sterility (CMS), the condition expressed in the lack of production of viable pollen grains[1]. According to them, the abortion of pollen in CMS plants occurred at almost every point in development and that probably more than one mechanism is involved. Anther ontogeny in an exotic and six indigenous cytoplasmic male sterile lines of wheat possessing *Triticum timopheevi* cytoplasm studied by JAIN *et al.*[7] has also shown breakdown of microsporogenesis at various stages. Present histochemical investigation has, therefore, been undertaken to study the nutritive function of tapetum during microsporogenesis in these CMS wheat lines along with their fertile counterparts.

## Materials and Methods

Cytoplasmic male sterility in seven indigenous varieties of wheat namely $HD_{1593}$, $HD_{2009}$, $HD_{2204}$, $K_{7435}$, $UP_{368}$, $WH_{157}$ and $HP_{1102}$ was introduced by crossing and backcrossing with an exotic CMS line 'Nadadores' obtained from U. S. A.

The spikes of male-sterile and male-fertile lines at different stages of development were fixed in 80% acetone. There were dehydrated, cleared and microtomed at 7-16 $\mu$m and HAUPT's adhesive was used for fixing the sections on the slides. The procedures adopted for various histochemical localizations as described by JENSEN[8] have been briefly described below:

*Total Carbohydrates of Insoluble Polysaccharides (Periodic Acid Schiff's (PAS) Reaction)*:

Deparaffinised sections were placed in 0.5% periodic acid for 20 minutes.

TABLE 1. Distribution pattern of PAS, DNA, histones and total proteins in the anthers of MF and CMS lines in various parts of an anther at different stages of development. The concentration of these substances has been arbitrarily divided into four parts viz. low or slight (+), moderate (++), high or intense (+++) and highest or most intense (++++)

| Reaction | Stages of development | Strain | Cuticle | Epidermis | Endothecium | Middle layer | Tapetum | Spore-mother cells | PMCs | Tetrad | Microspore | Pollen grains | Connective |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PAS | a | MF | | ++ | + | + | + | + | | | | | ++ |
| | | CMS | | + | + | + | + | + | | | | | + |
| | b | MF | | ++ | ++ | ++ | ++ | | ++ | | | | ++ |
| | | CMS | | ++ | + | + | ++ | | + | | | | + |
| | c | MF | | ++ | ++ | ++ | +++ | | | +++ | | | ++ |
| | | CMS | | ++ | + | + | ++ | | | ++ | | | + |
| | d | MF | +++ | +++ | ++ | ++ | + | | | | +++ | | +++ |
| | | CMS | | ++ | + | + | + | | | | ++ | | ++ |
| | e | MF | +++ | +++ | ++ | | | | | | | +++ | +++ |
| | | CMS | | ++ | ++ | ++ | | | | | | ++ | ++ |
| DNA and Histones | a | MF | | + | + | + | +++ | ++ | | | | | ++ |
| | | CMS | | + | + | + | + | + | | | | | + |
| | b | MF | | ++ | ++ | ++ | +++ | | +++ | | | | ++ |
| | | CMS | | + | + | + | + | | + | | | | + |
| | c | MF | | ++ | ++ | ++ | +++ | | | +++ | | | ++ |
| | | CMS | | + | + | + | ++ | | | + | | | + |
| | d | MF | | ++ | ++ | ++ | ++ | | | | +++ | | ++ |
| | | CMS | | + | + | + | + | | | | + | | + |
| | e | MF | | ++ | ++ | | | | | | | +++ | ++ |
| | | CMS | | + | + | | + | | | | | + | + |
| Proteins | a | MF | | + | + | + | +++ | +++ | | | | | ++ |
| | | CMS | | + | + | + | + | + | | | | | + |
| | b | MF | | + | ++ | ++ | +++ | | +++ | | | | +++ |
| | | CMS | | + | + | + | + | | + | | | | + |
| | c | MF | | + | ++ | ++ | +++ | | | +++ | | | +++ |
| | | CMS | | + | + | + | + | | | + | | | + |
| | d | MF | | + | +++ | + | ++ | | | | +++ | | ++ |
| | | CMS | | + | + | + | + | | | | ++ | | + |
| | e | MF | | + | ++ | + | | | | | | +++ | ++ |
| | | CMS | | + | + | + | + | | | | | ++ | + |

Stages (a) From archesporial initiation to formation of sporogenous tissue (Sporogenous tissue stage).
(b) Beginning of microspore mother cells to the end of meiotic karyokinesis (PMC stage).
(c) Cytokinesis partitioning the tetra nucleate microspore mother cell into microspore tetrad (Tetrad stage).
(d) Liberation of microspores from tetrads (Microspore stage).
(e) Maturation of pollen grains (Pollen grain stage).

After washing in distilled water for 10 minutes, they were stained for 30 minutes in SCHIFF's reagent. The sections were rinsed in water and placed in 2% sodium bisulphite for 1–2 minutes. After washing in water, they were dehydrated and mounted in euparal.

*DNA (Fulgen Reaction)*:

Deparaffinised sections were hydrolyzed in 1 N HCl at 58°–62°C for 15 minutes. After washing in distilled water the slides were placed in Schiff's reagent for 30 minutes at room temperature. The sections were rinsed in water placed in 2% sodium bisulphite for 1–2 minutes. These were washed in water, dehydrated and mounted. For control, alternate slides were placed in Schiff's reagent after extraction of nucleic acids in 15% trichloro-acetic acid at 100°C for 15 minutes.

*Histones (Alkaline Fast-Green Test)*:

Deparaffinised sections were placed in 15% trichloroacetic acid at 100°C for 15 minutes to extract the nucleic acids. After washing with three changes in 70% ethanol the sections were stained for 30 minutes in a 0.1% Fast Green (pH 8.0) at room temperature. The sections were dehydrated and mounted. For control alternate slides were kept in stain without extraction.

*Total Proteins (Ninhydrin-Schiff's Reaction)*:

After removal of paraffin, the sections were placed in 0.5% ninhydrin in absolute alcohol at 37°C for 24 hrs. Then they were rinsed in two changes of absolute alcohol and distilled water. After that the sections were placed in SCHIFF's reagent for 10–20 minutes at room temperature. For control, deamination and acetylation was done on alternate sections.

## Results and Discussion

The evaluation of PAS, DNA, histones and total protein in the anthers of various stages of development in MF and CMS plants has been presented in Table I. Anther development has been divided into five stages: (*a*) Sporogenous tissue stage, (*b*) Meiosis I and II stage, (*c*) Tetrad stage, (*d*) Microspore stage and (*e*) Pollen grain stage. The intensity of various reactions has been divided arbitrarily into four parts viz. low or slight (+), moderate (++), high or intense (+++) and highest or most intense (++++).

*PAS test*:

At sporogenous stage *a*, all the parts of an anther of MF lines exhibited slight reaction except epidermis, the cells of which were moderately stained. In the subsequent stages *b* and *c*, the intensity of the reaction gradually

increased (Fig. 1). Total carbohydrates of insoluble polysaccharides (TCIP) grains appeared in various wall layers including connective parenchyma. However, tapetum is devoid of starch at this stage (Fig. 1). On microspore tetrad formation (stage *c*), cuticle showed most intense, while intense reaction shown by the tapetal cells was well marked in their radial and inner tangential walls. Endothecial cells, however, showed only moderate reaction. At stage *d*, the intensity of the reaction remained more or less the same as in the previous stages with some fluctuation. The endotheical cells showed an increase from moderate to intense. At vacuolate pollen grain stage *e*, the intensity of the reaction increased further in all the layers except middle layer and tapetum degenerated by this stage and the engorged pollen grains showed highest concentration of TCIP grains (Fig. 2). The endotheical cells in the anthers at this stage also exhibited intense PAS reaction and characterstic fibrous bands also showed moderate reaction (Fig. 3).

On the other hand, the intensity of PAS reaction in the anthers of CMS plants either remained low or moderate at all stages and fell short as compared to those of MF lines (Fig. 4-7). TCIP grains also failed to accumulate in various wall layers and anther connective at any stage. However, some varietal fluctuations were also recorded in CMS lines. In CMS $HD_{1593}$, $WH_{157}$ and $K_{7435}$, the inner tangential walls of tapetum showed intense reaction at PMC and pollen grain stage (Figs. 7, 8). The cells on endothecium in the anther of CMS $HD_{1593}$ showed intense reaction at sporogenous tissue stage whereas it was inconspicuous in other lines. A limited number of TCIP grains were also seen in the anther connective at PMC stage in CMS $K_{7435}$ (Fig. 8).

The description on the localization of total carbohydrates of insoluble polysaccharides in anthers of MF and CMS plants indicated inconspicuous PAS reaction in sterile lines. These observations lend support to the findings of DE FOSSARD[6] in the anthers of *Chenopodium rubrum*. According to him, the development of endothecium is controlled by tapetum and only after the complete degeneration of tapetum, fibrous bands appear in the cells of this layer. In the CMS lines of wheat, the tapetal cells remain intact in most of the cases, and seem to inhibit the formation of thickenings in the endothecium. This fact is also supported by earlier findings of CHAUHAN[3]. Inconspicuous PAS reaction as a limiting factor for the growth of pollen grains in wheat has also been reported by JOPPA *et al.*[9] and CHAUHAN and RATHORE[5].

*DNA and Histones*:

The distribution pattern of histones and DNA in anthers of MF and

CMS lines was more or less parallel. The plausible reason for this may be due to 1 : 1 ratio of these substances[10].

At stage *a* various wall layers except tapetum exhibited low concentrations of DNA and histones, while the cells in tapetal layer showed intense reactions (Figs. 9, 12). The sporogenous cells also showed moderate reaction. The concentration of these substances increased with age and at stage *c*, it reached the peak being most intense in tapetum, intense in microspores in tetrads, while in the rest of the parts it was moderate or low. On the release of microspores from common callose wall, the intensity of the two reactions declined. However, with the growth of microspores, the concentration in them increased gradually with a steady decline in the tapetal cells which finally disappeared. The pollen grains exhibited highest concentration of DNA and histones (Fig. 11).

On the other hand, the concentration of these substances in various parts of an anther including malformed tapetum and pollen grains in various CMS lines failed to accelerate (Figs. 10, 13). These parts exhibited either low or moderate concentrations at all stages, indicating marked deficiency of DNA and histones. However, CMS $HD_{1593}$ exhibited some minor difference. The tapetal cells in the anthers of this line at stage *d* showed moderate reaction as against low in other CMS lines (Fig. 14).

Similar observations indicating deficiency of DNA and histones in CMS lines have also been recorded by BROOKS[2], ALAM and SANDAL[1], SAINI and DAVIS[14] and CHAUHAN and KINOSHITA[4].

*Total Proteins*:

The concentrations of proteins in various wall layers and sporogenous cells in MF lines was either low or moderate. A gradual increase in the concentration of proteins in various parts, tapetum and developing microspores in particular was recorded. The increase was maintained by vacuolate pollen grains which on becoming engorged with reserves, showed highest protein concentrations (Fig. 15). On the other hand, proteins in tapetal cells decreased as they were gradually used up.

Various CMS lines failed to exhibit an appreciable increase in protein concentration at any stage in various parts including intact tapetal cells and aborting pollen grains (Fig. 16).

All the CMS lines indicating deficiency of protein contents in the different parts of their anthers, which supported earlier findings[12,13,15].

On the basis of these observations it is concluded that the deficiency of the vital substances like DNA, histones and total proteins seems to serve as limiting factor for the development of viable pollen grains. This in all

probability was caused by the inhibiton of vascular supply in CMS anthers as suggested earlier by CHAUHAN and KINOSHITA[4] and JAIN *et al.*[7] in several cytoplasmic, genic as well as chemically induced male sterile plants including present CMS lines of wheat.


## Summary

A comparative histochemical studies in the anthers of cytoplasmic male-sterile lines of wheat (*Triticum aestivum* L.) var. 'Nadadores', $HD_{1593}$, $HD_{2009}$, $HD_{2204}$, $K_{7435}$, $UP_{368}$, $WH_{157}$ and $HP_{1102}$ and their fertile counterparts showed inconspicous PAS reaction reflecting marked deficiency of total carbohydrates of insoluble polysaccharides in the CMS lines. Such anthers were also significantly deficient in DNA, histones and total proteins. This in the opinion of the present authors was considered as the limiting factor for the growth of pollen grains which consequently aborted.



## Acknowledgement

Sincere thanks are due to Dr. Bahadur SINGH, Retired Scientist, C. S. I. R. for criticism, to Dr. S. N. CHATURVEDI, Reader and Head for encouragement, to Dr. Roshan SINGH, Principal for facilities and to Dr. D. L. KEIM for seeds. One of the authors (AKJ) is grateful to the C. S. I. R. for the award of Senior Research Fellowship.


## Literature Cited


1. ALAM, S. and P. C. SANDAL: Electrophoretic analysis anther proteins from male-fertile and male-sterile sudan grass *Sorghum vulgare* L. var. sudanese (Piper). *Crop Sci.* **9**: 157-159. 1969
2. BROOKS, M. H.: Nucleic acid studies of feritle, cytoplasmic male sterile and fertility restorer lines of *Sorghum vulgare*. *Sorghum Newsletter*. **8**: 53-54. 1965
3. CHAUHAN, S. V. S. Development of endothecium in relation to tapetal behaviour in some male sterile plants. *Phytomorphology*. **29**: 245-251. 1979
4. CHAUHAN, S. V. S. and T. KINOSHITA: Histochemical localization of histones, DNA and proteins in the anthers of male-fertile and male-sterile plants. *Japan. J. Breed.* **29**: 287-293. 1979
5. CHAUHAN, S. V. S. and RAJESH, K. S. RATHORE: Histochemical evaluation of PAS reaction in the anthers of male-fertile and male-sterile plants. *Curr. Sci.* **49**: 433-436. 1980
6. DE FOSSARD, R. A.: Development and histochemistry of the endothecium in the anthers of *in vitro* grown *Chenopodium rubrum* L. *Bot. Gaz.* **130**: 10-22. 1969
7. JAIN, A. K., R. K. S. RATHORE, S. V. S. CHAUHAN and T. KINOSHITA: Anther ontogeny in an exotic and six indigenous cytoplasmic male sterile lines of wheat

(*Triticum aestivum*) possessing *Triticum timopheevi* cytoplasm. *Japan J. Breed.* **31** (3): 251–260. 1981

8. JENSEN, W. A.: Botanical histochemistry. W. H. Freeman and Co., San Francisco. p. 408. 1962
9. JOPPA, L. R., F. H. McNEAL and J. R. WELSH. Pollen and anther development in cytoplasmic male-sterile wheat (*Triticum aestivum* L.). *Crop Sci.* **6**: 296–297. 1966.
10. KNOX, R. B. and L. T. EVANS. Australian J. Biol. Sci., **19**: 235–245. Ref. Heslop-Harrison, J. 1967. Differentiation. *Ann. Rev. Plant Physiol.* **18**: 325–348. 1966
11. LASER, K. D. and N. R. LERSTEN: Anatomy and cytology of microsporogenesis in cytoplasmic male sterile angiosperms. *Bot. Rev.* **38**: 425–454. 1972
12. MIAN, H. R., G. W. R. WALKER and N. MUNTJEWERFF: Histological and cytochemical studies on five genetic male-sterile lines of barley (*Hordeum vulgare*). *Can. J. Genet. Cytol.* **16**: 355–379.
13. NAKASHIMA, H. and S. HOSOKAWA: Histochemical studies on the cytoplasmic male sterility of some crops. II. Changes of DNA, polysaccharides and proteins and translocation of photosynthetic products of sugar beets. *Mem. Fac. Agr. Hokkaido Univ.* **8**: 1–4. 1970
14. SAINI, S. S. and G. N. DAVIS: Male sterility in *Allium cepa* and some species hybrids. *Econ. Bot.* **23**: 37–49. 1969
15. TRIPATHI, D. P., S. L. MEHTA and G. P. RAO: Soluble protein and isoenzymes from anthers of diverse male steriles in sorghum. *Indian J. Genet.* **41** (I): 170–177. 1981

## Leged for Plate I

Figs. 1-8. Transverse part of anthers of MF and CMS lines showing PAS reaction.

Fig. 1. MF, PMC stage. Note the presence of TCIP grains. 310×.

Fig. 2. MF, Engorged pollen stage. 110×.

Fig. 3. MF, Magnified view of one of the anther lobes shown in Fig. 2. Note the presence of fibrous bands in endothecial cells. 340×.

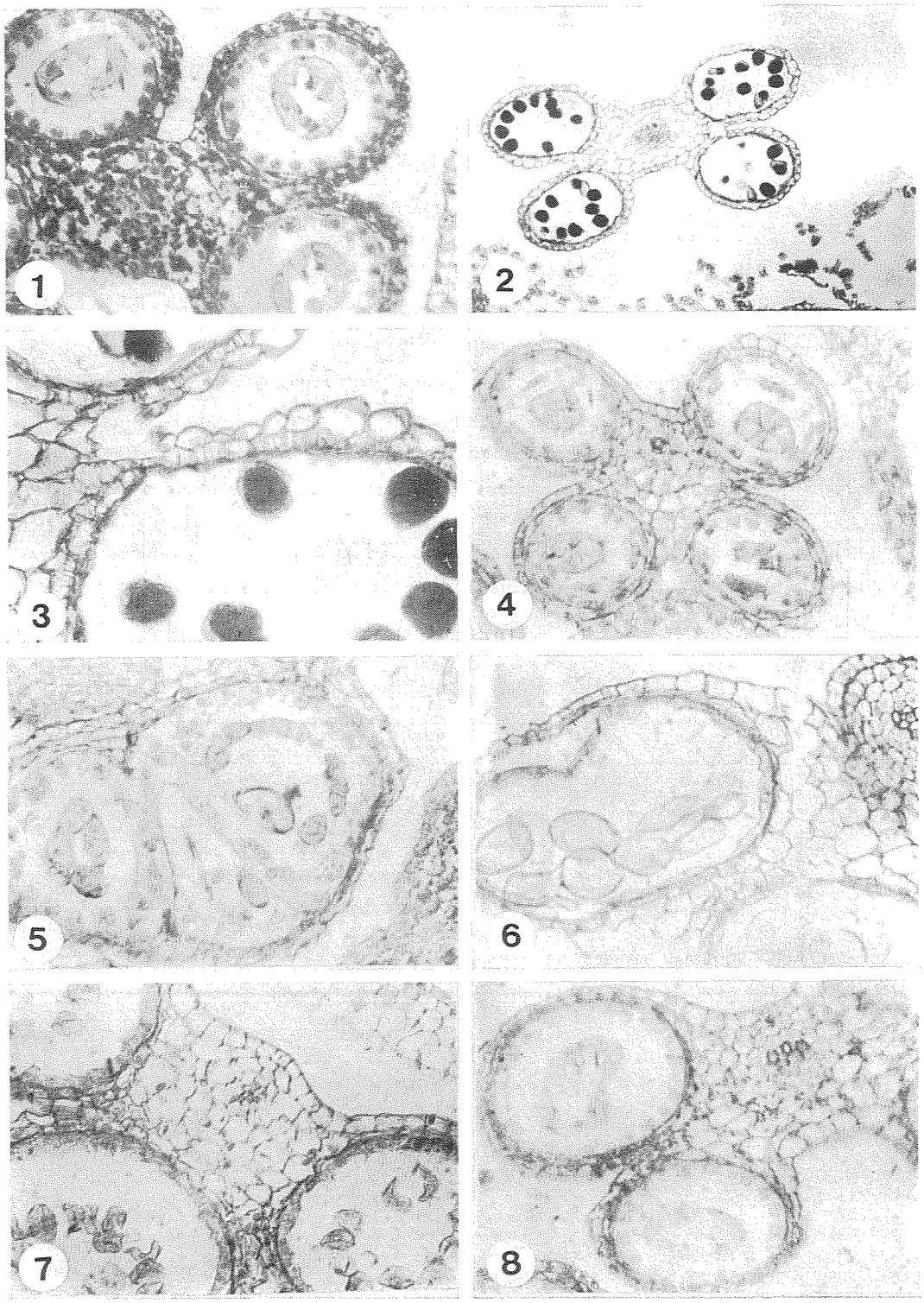
Fig. 4. CMS, $HD_{2009}$ sporogenous tissue stage. Note the absence of TCIP grain. 310×.

Fig. 5. CMS, $HD_{2204}$ Microspore stage. Note tapetal hypertrophy. 340×.

Fig. 6. CMS, $UP_{368}$ Pollen grain stage showing poor PAS reaction. 310×.

Fig. 7. CMS, $HD_{1593}$ Pollen grain stage. Note intense PAS reaction in the inner tangential tapetal walls. 340×.

Fig. 8. CMS, $K_{7435}$ PMC stage. Note the presence of few TCIP grains in anther connective with poor vascular tissue. 310×.


1
2
3
4
5
6
7
8

## Legend for Plate II

Figs. 9–14. Transverse part of anthers of MF and CMS strains showing localization of DNA and histones.

Fig. 9. MF, DNA localization at sporogenous tissue stage. 310×.

Fig. 10. CMS, $HD_{2009}$ DNA localization at sporogenous tissue stage. 310×.

Fig. 11. MF, Histone localization at pollen grains stage. 310×.

Fig. 12. MF, Histone localization at sporogenous tissue stage. 280×.

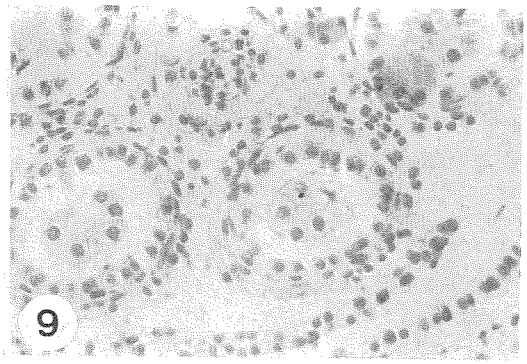
Fig. 13. CMS, $UP_{368}$ Histone localization at microspore stage. 310×.

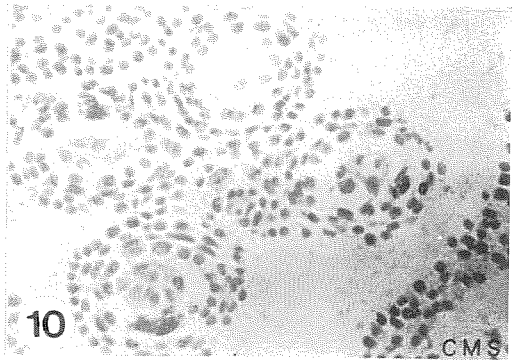
Fig. 14. CMS, $HD_{1593}$ Histone localization at microspore stage. 310×.

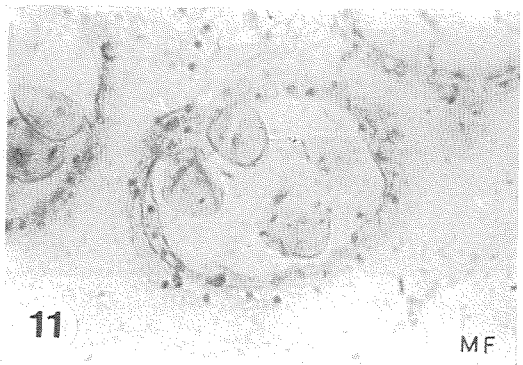
Figs. 15–16. Transverse part of anthers of MF and CMS lines showing protein localization.

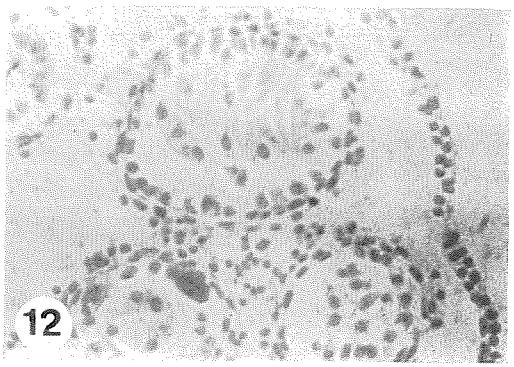
Fig. 15. MF, Pollen grain stage. 340×.

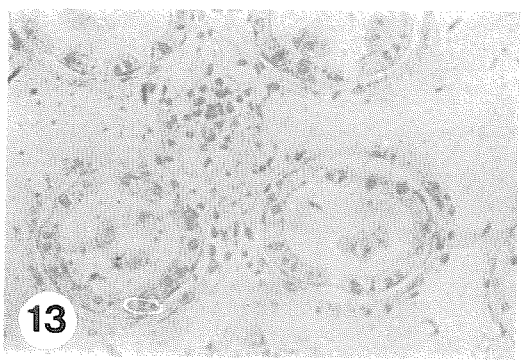
Fig. 16. CMS, $UP_{368}$ Pollen grain stage showing low protein contents. 310×.

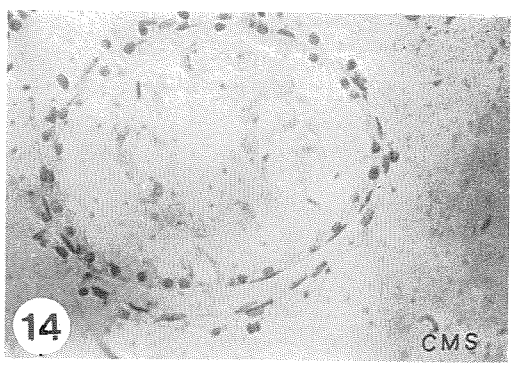

9

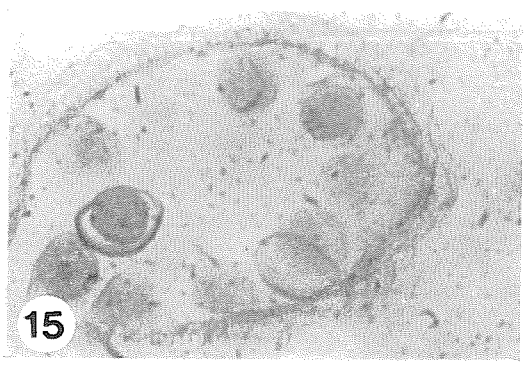

10
CMS

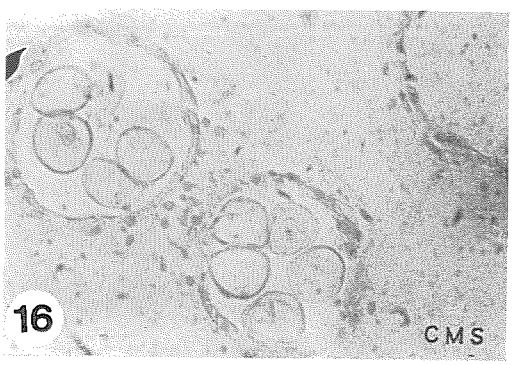

11
MF


12


13


14
CMS


15


16
CMS