Summary

Conclusion

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HISTOLOGY

The biological pattern of microsporogenesis in fertile and sterile (CMS) anthers of *Raphanus* and *Pennisetum* is similar up to the release of microspores from tetrads. In CMS *Raphanus* anther, the first sign of abnormality is noticed in tapetum, at just released microspore stage, which forms syncytium. Subsequently, tapetal syncytium and these microspores begin to degenerate and collapse finally. At times hypertrophy of tapetum was also noticed. In such instances of latter kind, tetrads themselves are crushed and collapsed.

In CMS *Pennisetum*, unlike in its fertile anther and that of *Raphanus*, tapetum continues to persist. This indicates, a striking difference between the fertile and sterile anther of *Pennisetum*. Excepting this, in both *Raphanus* and *Pennisetum* no other structural differences were observed in the growth and function of wall layers.

HISTOCHEMISTRY

Total insoluble polysaccharides, ascorbic acid (AA), RNA, DNA, and proteins were localized by using fixed material sections, during successive stages of microsporogenesis of both fertile and sterile plants of *Raphanus* and *Pennisetum*. Histochemically, no significant differences have been observed between sterile and fertile anthers of these plants until completion of microspores stage is reached. This feature is obvious because of the fact that pollen abortion in sterile plants takes place during post meiotic development of the anthers only.

In the present study, role of callose is also not very crucial because of its normal occurrence around meiocytes and tetrads as a regular feature, and its dissolution is also normal in both fertile and sterile lines of *Raphanus* and *Pennisetum*.
Microspores released from tetrads generally contain considerable amounts of RNA, ascorbic acid, proteins in fertile anther, but not even so in the sterile anthers. These visible differences in cellular contents suggest the possibility of hampered metabolic activities during the formation of pollen grains in sterile anthers. While in both the plants, fertile anthers show accumulation of starch in the viable pollen grains, in the sterile ones, on the contrary, microspores do not have starch and as a result they degenerate. Therefore general lack of starch in the microspores and pollen indicates nonviability.

The functional behaviour of tapetum in both fertile and sterile anthers of *Raphanus* and *Pennisetum* is differential. In the fertile anther of both the plants the tapetum generally maintains high levels of RNA and proteins and also AA up to microspore stage indicating its normal biochemical activity and function all through meiosis. This vital tissue when functions generally do not contain storage PAS grains, But, rich PAS positive stain prevails in *Raphanus*. Even minimal amount of lipids have also been noticed in the tapetum. In CMS *Raphanus*, following tetrad formation, the tapetum becomes vacuolated and gradually develops tapetal syncytium showing considerable activity for RNA and proteins but low synthesis of AA. The latter observation is in contrast to that of fertile anthers in *Raphanus*. In this plant rarely tapetum also becomes hypertrophied and reacts considerably positive to RNA, proteins and AA. The tissue also reacts strongly to PAS test particularly more towards locular side of the enlarged tapetal cells. Although the tapetal syncytium also shows hypertrophied nature, and the latter, however, invariably is involved in crushing the tetrad of microspores. Therefore, hypertrophy or syncytium of tapetum appears to be a visible morphological cause for the abortion of microspores in *Raphanus*. It is shown here that any morphological change of this kind is concomitant with biochemical changes which occur at molecular levels.

This may be true in the present context. In CMS *Pennisetum* also, activities of these substances, namely RNA, proteins and AA decline in microspores, but on the contrary they persist in tapetum for considerable period in contrast to its fertile
line. This period of tapetal activity in sterile anthers may be due to biochemical and structural impairment causing visible functional disturbance causing sterility. It is not clearly known whether this happening is a cause or effect which, brings about abortion of pollen. In CMS Pennisetum, anthers show considerably low activity for RNA, proteins and AA in the persistent tapetum. Which is contrast to its fertile anthers. However, the tapetum here, as it appears not involved in causing sterility. The only difference perceived between CMS and fertile anthers is the delayed degeneration of tapetum in the former. Whether this difference exerts any influence in causing sterility of microspores or not is yet to be elucidated. It is possible that microspores themselves collapse in the locule due to their internal happenings and might lead to sterility. Therefore, it is not known whether the cause of sterility lies in the microspores or even in the tapetum. The probably cause(s) for sterility can be studied, if one analyses the individual tissues by biochemical procedures.

In the normal anthers, anther wall layers show storage of abundant starch in Raphanus and Pennisetum until the formation of microspores. Subsequently, starch storage is depleted. Consequently, differentiation of endothelial thickenings and accumulation of starch in the developing pollen takes place. There appears to be some kind of nutritional correlation between the starch disappearance and appearance of it again in the pollen coupled with the differentiation of endothelial thickenings. In the sterile anthers of the both Raphanus and Pennisetum on the contrary, the depletion of storage starch is observed in microspores and however, no formation of endothelial thickenings. Therefore, it appears that the endothecium does not take part in causing male sterility. Middle wall layers in these plants also contain considerable PAS and AA grains up to microspore stage, subsequently the storage declines and lost.
HISTOENZYMOL OGY

As needed, cryostate sections of fresh anthers are used for localization of enzymes. They are: glucose-6-phosphate dehydrogenase, succinate dehydrogenase, glutamate dehydrogenase, Malate dehydrogenase, isocitrate dehydrogenase, acid phosphatase, alkaline phosphatase, ATPase, peroxidases, cytochrome oxidases, esterases and lipids. In fertile lines of *Raphanus* and *Pennisetum* higher activities of the dehydrogenases and cytochrome oxidases are observed in tapetum at microspore stage, and a parallel increase in the activities of these in the microspores and pollen grains. On the contrary in the in CMS anthers of *Raphanus*, reduced enzyme activity was noticed in syncytium tapetum and also in the degenerating microspores. In CMS *Pennisetum* also, low activity is observed in persistent tapetum and degenerating microspores which indicates that the reduction of enzymatic activity corresponds to the reduction in number of mitochondria. The reduction of mitochondrial enzymes is quite significant indicating the possible disturbances of respiratory activities in the tapetum and microspores of CMS anthers. High activity of acid phosphatase, alkaline phosphatase, ATPase is observed in the tapetum at microspore stage until the degeneration of tapetum. These happenings reflect involvement of enzymes in the formation of exine, and also suggest the much needed storage nature of fertile pollen. In male sterile anthers degenerating microspores lack high activities of these enzyme causing poor formation of exine or lack of it.

In middle wall layers of fertile anthers of *Raphanus*, activities of peroxidases and esterases are strong when compared to those of sterile ones. Therefore, the middle wall layers of both sterile and fertile lines seem to play equally an important physiological role in hydrolysis of stored substances in them.
BIOCHEMICAL WORK

Using polyacrylamide gel electrophoresis technique, differences in protein profiles between fertile and CMS lines of *Raphanus* and *Pennisetum* have been studied. The results revealed the occurrence of certain additional protein bands in fertile anthers when compared to similar situation in sterile ones. This observation apparently is in line with the reported synthesis of gametophytic proteins in fertile anthers. The results obtained in the present study have been discussed in the light of relevant previous work.