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Cytogenetical and Developmental Studies of Male Sterility in Brassica, Campestris L.

Hossain, Mrs. Salma
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CYTOGENETICAL AND DEVELOPMENTAL STUDIES OF MALE STERILITY IN *Brassica campestris*

A

Thesis submitted for the degree of
Doctor of Philosophy
in Botany
of the University of Rajshahi

By

Salma Hossain, M. Sc., M. Phil.

Department of Botany
University of Rajshahi, Rajshahi 6205
Bangladesh,
Year- 1991

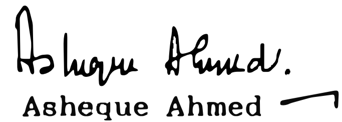
Dedicated

to

My Beloved Parents

CERTIFICATE

This is to certify that the thesis entitled "Cytogenetical and developmental studies of male sterility in *Brassica* campestris L." is an original work done by Mrs. Salma Hossain for the degree of Doctor of Philosophy in Botany. The style and contents of the thesis have been approved and recommended for submission.



Asheque Ahmed
Professor, Department of Botany
University of Rajshahi.

ACKNOWLEDGEMENT

The author expresses her deep sense of gratitude to her supervisor Prof. Asheque Ahmed for guidance and supervision.

She is indebted to the Chairman and other teachers of the Department for their help and encouragement.

She expresses her thanks to Mr. A. M. N. Chowdhury and Mr. G. P. Ghosh, Research Fellow of the Department for their help. Thanks are also due to Dr. R. H. Sarker, Assistant Professor of Botany, Dhaka University for his help.

She is indebted to Prof. M. R. Bijayraghabhan of the Department of Botany, University of Delhi, and Prof. H. G. Dickinson, Plant Science Laboratories, University of Reading, for their comments and help.

She is grateful to the Division of Science and Technology, Ministry of Education, Government of Bangladesh for granting her a Senior Research Fellowship for three years to carry out the research.

The author thanks Mr. M. I. Waheed for Computer-composing the thesis with utmost care.

The author

ABSTRACT

The inheritance of male sterility and comparative study of the development of male sterile and male fertile anthers of *Brassica* were carried out.

Male sterility in one family of *B. campestris* Var. Toria and a family of *B. campestris* Var. Yellow Sarson both were found to be controlled by single recessive genes.

The development of anther in *Brassica* was found to follow the typical dicotyledonous tetrasporangiate type. The details of the developmental stages are described.

Comparative study of microsporogenesis in *Brassica campestris* L. Var. Toria and Var. Yellow Sarson indicated four different types of development of male sterility :

(a) abnormal behaviour in the sporogenous cells before the initiation of meiosis followed by the breaking down of tapetum;

(b) abnormal behaviour initiated due to the persistence of tapetum long after meiosis resulting in the abortion of microspores or tetrads;

(c) abnormal behaviour in the early degeneration of young tapetal cells followed by break down of meiocytes; and

(d) abnormal behaviour of tapetum before the onset of meiosis in hypertrophied growth of young tapetal cells crushing the meiocytes.

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ABBREVIATIONS

BC	*Brassica campestris*
CMS	Cytoplasmic Male Sterility
FAO	Food and Agriculture Organisation
FFYP	Fourth Five Year Plan
GMS	Genetic Male Sterility
MF	Male Fertile, Male Fertility
MMC	Microspore Mother Cells
MS	Male Sterility, Male Sterile
OP	Open Pollinated
PMC	Pollen Mother Cell
SC	Self Compatible
SI	Self Incompatible
T	Toria (var.)
YS	Yellow Sarson (var.)

INTRODUCTION

1.1. The background

Brassica is the most important oil seed producing genus of the family Cruciferae. In the Indo-Pak-Bangladesh Subcontinent, there are three ecotypes of *Brassica campestris* L. ssp. oleifera (2n=20) : Toria, Yellow Sarson and Brown Sarson. Toria is the most commonly cultivated ecotype evolved in the foot-hills of the Himalayas and in the plains of Bangladesh and West Bengal. This is brown-seeded, early maturing, drought tolerant and is sown in September and matures by late December. Whereas Yellow Sarson has restricted distribution with whitish yellow seeds and is late maturing. Brown Sarson is not found in Bangladesh but is cultivated widely in Central India. Another species, *B. juncea* grown as a late crop for edible oil in this area is an allotetraploid (2n=36).

In Bangladesh, rapeseed (*B. campestris*) and mustard (*B. juncea*) are by far the most important in terms of production and share of total oil crop area. During 1989-90, a total of 586,815 ha were under oil seed crops producing 464,000 tons of which 330,000 tons were edible oil seeds. Of these, rapeseed and mustard covered about 65 percent of oil crop area.

Domestically, about 80,000 tons of edible oil was produced in 1982-

83 which was 45 percent of the estimated requirement. Thus, Bangladesh depends for more than 55 percent of edible oil from foreign import (Ahmed, 1988). During 1984-85, 136,000 tons of edible-oil worth 103 million US$ and 28,000 tons of oil seed worth 11 million US$ were imported. During 1989-90, the actual import of edible oil was increased to 425,000 tons with a cost of about 300 million US$ and that of oil seed was 50,000 tons with an expense of 16 million US$. According to the Fourth Five Year Plan (FFYP) Report, edible oil import projected for 1994-95 is 525,000 tons and oil seed import is projected to rise to 100,000 tons.

The area and production of rape seed and mustard in Bangladesh over the period of 1947-88 is given in Fig. 1.1. It is evident that both the acreage and production stagnated and even declined; the overall increase in production was only 0.7 percent per annum during 1965-81. Between 1982-88 there was a slight rise in production partly due to introduction of several high-yielding varieties (HYV). The yield of rapeseed is only 600 to 650 kg/hectare which is very low. If the yield in Bangladesh over the years is compared with that of a developed country like Sweden (Fig. 1.2) the trend in yield in these two countries become apparent.

In India, too, improving productivity of oil seeds assumes importance in the present context of acute shortage in the availability

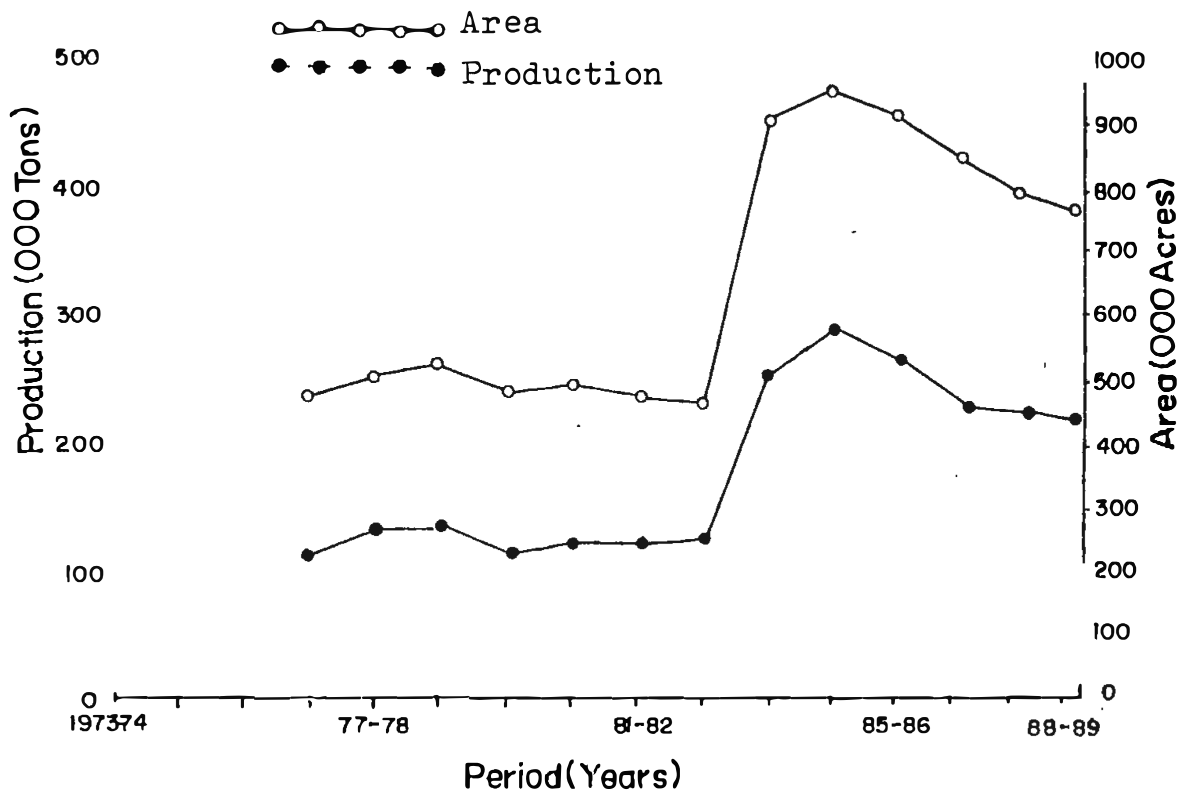

Fig 1.1 Area and production of rapeseed and mustard in Bangladesh during 1973-74 to 1988-89

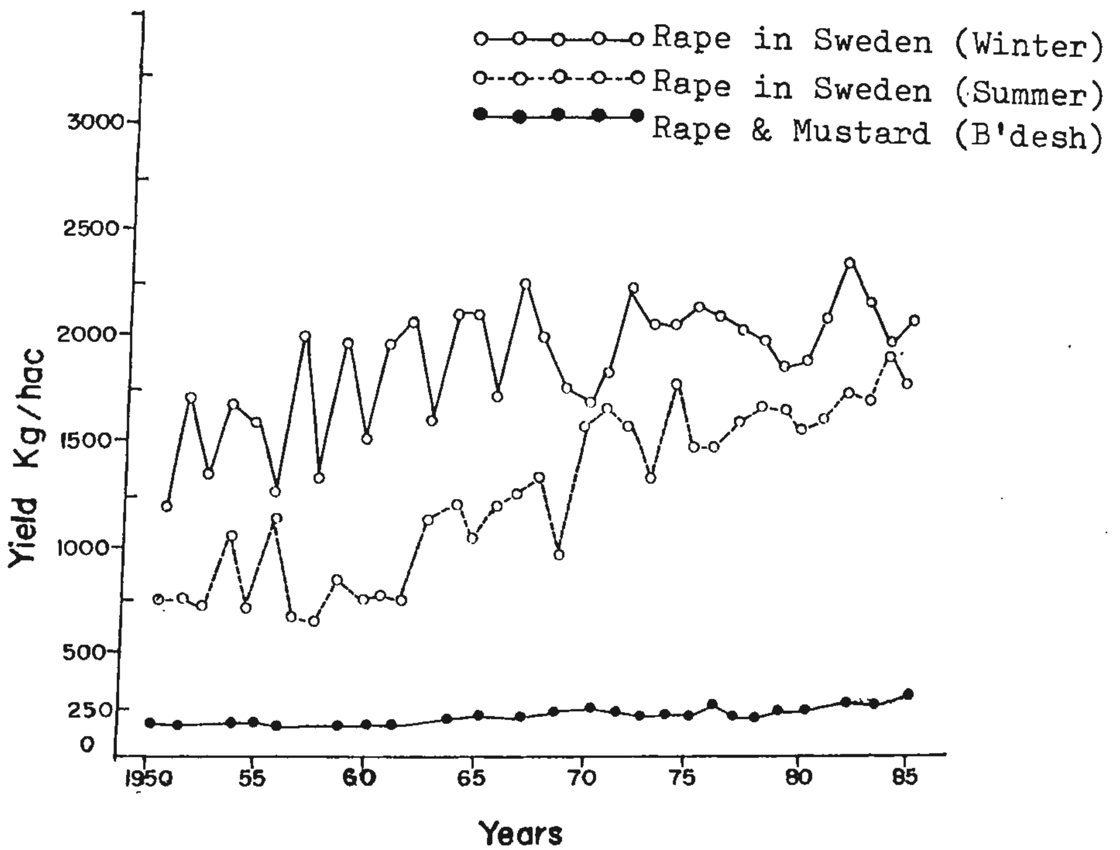

Fig 1.2 The yield of rape and mustard in Bangladesh and in Sweden

of edible oils (Prakash and Raut, 1983). Next to ground-nut, *Brassica* is the main oil-seed crop of India but a national average of 600 kg/hectare is one of the lowest in the world. India produced 2.2 million tons of rapeseed from 4.06 million hectares in 1981, whereas Canada and Sweden produced 1.7 and 0.35 million tons from 1.4 and 0.17 million hectares respectively (FAO Production Year Book, 1982).

It may be noted that oils and fats are very important component of daily diet. An average Bangladeshi should take 40 gm oils and fats daily. The present level of availability of oils and other food items are given in Table 1.1. It is obvious, how critical is the scarcity of vegetable oils in the diet of the people of this country. Too low an intake of fat and oil limits the availability of fat-soluble vitamins and causes dietary imbalance and wastage resulting in poor growth and development. Vegetable oil is the main source of fat and in the average Bangladesh diet, there is an acute shortage of this item now.

Growing cereals as major crops will gradually become more expensive and will require more fertilizers, insecticides, and fungicides and thus will increase pollution. Crop diversification and cultivation of crops resistant to pests, diseases and requiring less fertilizer and irrigation will get more emphasis. Thus, the importance of crucifer-crops as oil seed will certainly increase specially due to erratic supply and increased expense of mineral and animal oil.

Table 1.1 : The production and Requirement (Per capita per day) of food components in Bangladesh.

Component	Production (g)	Requirement (g)	Deficiency (%)
Cereals	397	408	3
Pulses	5	58	91
Sugar	17	29	41
Oil	2	15	87
Milk	25	58	57
Meat	10	15	33
Fish/egg	17	29	41
Vegetables	61	233	71
Fruits	21	29	28

FFYP Report, 1990.

Per unit area per unit time of the *Brassica* crop in terms of utilizable energy and protein is much higher than cereals. It is not unreasonable to foresee an expansion of oil seed *Brassica* in the next few decades. As a source of animal feed more crucifers will be used in future and their importance will increase further.

Several other advantages exist in favor of cultivating rapeseed in this Subcontinent fitting very suitably to the cropping system : (a) it contributes to a better crop rotation fitting between two major rice crops, (b) it results in a better distribution and utilization of the agricultural labour in the lean period, (c) it is a much preferred crop to the poor, marginal farmers requiring little modern and expensive farming technology, (d) it has a very short growing season of about 80 days utilizing the residual moisture very efficiently in the dry winter season, and (e) the rich alluvial soil is suitable for this crop after the flood water recedes without elaborate land preparations.

All the above considerations point to the increase in rapeseed productions very urgently. The FFYP promises to give determined efforts to increase oil seed production to 6.4 lakh tons from the present level of production of 4.6 lakh tons by 1994-95. According to the Plan, strong support will be given in the form of intensive research efforts for HYV's and for intensive work to increase the area under oil seeds (FFYP Report, 1990, Page Va-35).

But as Bangladesh is a country with an already very intensive land utilization and there is no land left for expansion of area under rapeseed and in view of the very low yield per hectare, the only possibility of reaching self-sufficiency is through increasing yield per unit area by plant breeding and better crop production techniques.

1.2. *Brassica* breeding

In countries like Canada, Australia, U.K., Sweden and Germany, a number of HYV's of *Brassica* crops were bred and released which along with better husbandry and management have resulted in much higher yield (Fig. 1.2). But in the Indian subcontinent, attempts for improvement of this crop by mass selection and synthetic varieties were of very limited success. Breeding through genetic improvement of *Brassica* crops has been going on since early 1930's in this Subcontinent. For self-pollinated and self compatible crops of *B. juncea* and Yellow Sarson, pure line selection was used to improve yield (Muhammad, 1935; Muhammad and Sikka, 1941). Mass selection was employed for cross-pollinated and self-incompatible cultivars of Toria and Brown Sarson. Very little success could be achieved following these attempts. Afterwards these breeding methods were supplemented with polyploidy (Prakash, 1970, 1973), mutation breeding (Basu *et al.*, 1970) and mass-pedigree selection with only 10 to 20 per cent increase over the existing cultivars in India. During 1960's and 1970's, in

Pakistan and in Bangladesh, increased production was obtained mostly by improved cultural practices and by increasing the area under cultivation. The better varieties selected were not very successful and were adopted to very limited areas. Thus the "green revolution" failed to improve rapeseed production in this region. Though several HYV's like Sonali Sarisa, Sampad, Kalyani were released during last few years, they require improved crop husbandry techniques which the poor farmers can not supply. Another serious difficulty is the rapid genetic break drown of the HYV's due to widespread cross pollination which always takes place in these out-breeding crops. The Rabi Season, during which the crop is grown is characterized in Bangladesh by low and very aberrant rainfall and temperature fluctuations. The entire crop is raised under rainfed condition with very low levels of inputs. Under these conditions, the HYV's with a requirement of better management and husbandry give very poor performance; the local varieties and land-races do better without high investment though their yield is very low.

1.3. Hybrid varieties and *Brassica* breeding

To increase the yield per unit area, production of improved varieties of *Brassica* is very important. To overcome the existing limitations in *brassica* breeding as mentioned in the previous section new

breeding techniques are to be applied to improve production.

Attempts to introduce *B. napus* (2n=38) to Bangladesh has been considered by Wahiduzzaman (1987). The prospect of *B. napus* in Bangladesh is not very bright because of its longer growing period, higher degree of shattering of the fruits, sterility and pod abortion.

It is most appropriate at this stage to consider another new method for improvement of yield of oil seed *Brassica* in Bangladesh i.e. the possibility of using hybrid varieties. Hybrid varieties have many important advantages over the conventional HYV's i.e. they produce higher yield, better quality and uniform crops with uniform maturity. They are disease resistant and require less investment. Various components of yield have been found to be subject to heterotic vigour in *Brassica* (Rajan, 1970; Rao, 1970; Singh and Singh, 1972). The hybrid varieties could be produced in place of the present mass-selected varieties. The possibility of using hybrid varieties to get higher yield is bright in view of the success of single-cross, double-cross, and triple-cross hybrids of *Brassica* bred and introduced in Europe.

The first reported commercial production of F_1 *Brassica* was a cabbage cultivar in Japan in 1938 (Sakata, 1973). Hybrid seeds are produced in a large variety of crucifer-vegetables in Japan, USA and in a wide range of European countries.

Johnston (1963) and du Crehu (1969) showed that F_1 hybrid *B.*

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oleracea (Kale) greatly out-yielded open-pollinated cultivars. Double-cross and triple-cross hybrids using four and six inbred lines, respectively, were employed to produce commercially profitable hybrid seeds (Thompson, 1964). The first produced hybrid kale is Maris kestrel, which is a double-cross, super seded by a triple-cross in 1975 (Thompson, 1971).

Many of these hybrid varieties were produced using self-incompatibility. A problem associated with the F_1 seeds utilizing self-incompatibility is that parental materials have low seed yields due to pronounced inbreeding depression. This problem is more important in the oil-seed crops like rapeseed than in the vegetable crucifers (i.e. cabbage, cauliflower, broccoli etc.). Another problem is that self-incompatibility may be weakened due to inbreeding or by fluctuations in the environment (i.e. temperature, rainfall, humidity), thus some selfed seed may be produced along with hybrid seeds (Johnson, 1966).

These problems have pointed out to other methods of producing F_1 and hybrid seeds. Use of male sterility is one of them.

Progress with the production of F_1 as well as other hybrid *Brassica* highly depends on a detailed knowledge of the reproductive biology and breeding system. Such research are mostly restricted to the temperate regions, no such work has been done in the tropical and subtropical countries like Bangladesh.

1.4. Male sterility and *Brassica* breeding

In flowering plants, the term male sterility (MS) indicates the absence or non-functioning of male gametophyte or pollen grains in a bisexual flower. Male sterility may result from interspecific or intergeneric hybridization, chromosomal aberrations, gene action or cytoplasmic influence. The term MS can and does mean a number of situations. Many different types of MS (Male-sterility) are thus reported; sterility due to morphological reasons are :

a) Absence (due to abortion) or extreme paucity of pollen grains in anthers of bisexual flowers (pollen sterility);

b) Non-functional, malformed or completely lacking of stamens or male flowers (Staminal sterility);

c) Non-dehiscence of anthers with functional pollen grains (Functional sterility).

All these results in the prevention of effective pollination in bisexual flowers. Thus, in nature, MS is an undesirable phenomenon and render a lowered fitness to its bearer and is generally eliminated by natural selection.

Male sterility in flowering plants is potentially useful for both the plant breeders and the botanists. The former use the MS lines

in breeding programmes. Specially in breeding hybrid and improved varieties, MS has been proved very useful, because in the MS plants it is possible to make cross pollinations without the laborious task of emasculating each flower. Whereas to the botanists, MS lines are excellent experimental materials for investigating the mechanisms involved in pollen and stamen development.

In the past, emphasis was placed by research workers on studying the inheritance of the MS for hybrid production. Much less is known about the development and the ontogenetic processes in pollen abortion. Such studies are essential for fully understanding the mechanisms involved in MS, which is advantageous for its utilization in breeding programmes.

Male sterility determined by a single gene has been identified in several cruciferous crops.

In *Brassica campestris* Var. Yellow Sarson, MS was reported by Chowdhury and Das (1967a). They also reported functional MS in Brown Sarson (Chowdhury and Das, 1967b). Koch and Peters (1953) reported genetic MS in *B. napus*. In *B. napus*, Takagi (1970) induced a recessive MS mutation with gamma radiation. Chowdhury and Das (1967b) reported non-allelic genes for MS in self-incompatible and self-compatible cultivars of Brown Sarson in India.

In *B. oleracea* MS genes have been reported in cabbage (Nieuwhof, 1961), in Brussels spouts (Johnson, 1958; North, 1961; Nieuwhof, 1968), in cauliflowers (Jensma, 1957; Nieuwhof, 1961; 1968; Borchers, 1966) and in calabrese (Cole, 1957; Sampson, 1966, 1970; Dickson, 1970; Borchers, 1971). The present author reported MS in *B. campestris* L. Var. Toria (Zuberi *et al.*, 1981, Zuberi and Zuberi, 1983).

. Male sterile material could be bulked up by micro-propagation and the feasibility of this method has been shown for cauliflower, a species of *Brassica* (Crisp and Walkay, 1974).

REVIEW OF LITERATURE

2. Review of Literature

The first report of male sterility in crop plants was made by Bateson and Gairdner (1921) in flax. Since then male sterile plants have been reported in many other crops such as corn (Rhodes, 1933), onion (Jones and Ensweller, 1936), sugar beets (Artschwager, 1947), tomato (Roever, 1948), carrots (Welch and Grinbell, 1947) lima beans (Allard, 1953) etc.

Male sterility may either be nuclear or genic (GMS), or cytoplasmic (CMS), or it may involve both the nuclear and cytoplasmic genes. In the present study only GMS type of MS will be considered and the term MS will be used in place of GMS.

In *Melilotus alba*, Casteller (1925) reported a MS which resulted due to cytokinesis failure after meiosis. The extent of cytokinesis failure varied in the microspore mother cells (MMCs) of a given locule.

Rhodes (1933), in *Zea mays* studies the differences between the pollen of male sterile and male fertile plants. Artschwager (1947) reported a periplasmodial tapetum in cytoplasmic male-sterile *Beta vulgaris*. The semi-sterile anthers, however, showed no periplasmodium but only a hypertrophied tapetum.

Rick (1945) observed high frequency of *Ms* genes in tomato and reported at least 18 separate pollen sterility genes in this crop (Rick,

1956). The breakdown was reported to occur at many different stages from the premeiotic stage to the late microspore stages.

A functional pollen sterility was reported in tomato by Larson and Paur (1948) which was controlled by a recessive gene. The sterile plants produced viable pollen but the stomia of the anthers failed to open, thereby the pollen was prevented from reaching the stigma. It was also regarded that the interaction between tapetum and sporogenous cells is the cause of sterility in a number of seed plants.

Rick and Robinson (1951) reported a tomato mutant with vestiges of stamens present in pistillate mutant with complete absence of pollen. The gene *Pi* renders high ovule sterility in the MS plants too.

A pollen sterile lima bean was described by Allard (1953). The ***Ms*** gene was found to affect the first true leaf of the plant. Shukla (1954) and Dnyansagar (1954) noticed a tapetum intermediate between secretory and amoeboid type in ***Lens esculentum*** and ***Adenanthera pavonina,*** respectively.

Bishop (1954) described a stamenless mutant in tomato with vestiges of stamens adherent to pistils. This gene ***sl*** genetically controlled this phenotype and was female fertile.

Jasmin (1954) reported a functional pollen sterile eggplant where pollen was produced by anthers failed to dehisce thus barring normal pollination.

Singh and Hadley (1961) reported a multinucleate persistent, lightly stained tapetum in male sterile sorghum. Zenkteler (1962) reported a persistent tapetum in ***Daucus carota*** resulting in MS.

Singh and Sharma (1963) studied in male sterile ***Pennisetum,*** factors responsible for germination, survival of seedlings, green-ear attack, and smut disease. Webster and Singh (1964) found that in a ***Sorghum*** variety with non-dehiscent anthers, there was delayed degeneration of tapetum leading to sterility. The same cause was attributed to the sterility in flax by Dubey and Singh (1965). Erichson and Ross (1963) studied colchicine induced male sterile mutant in sorghum and reported the cause of sterility as genetic disturbances in pollen grains and hypertrophied or persistent tapetum.

Joppa ***et al.*** (1966) observed, in male sterile wheat anthers, poorly differentiated vascular bundle resulting in reduced transport of metabolites leading to lack of starch in the microspores. Grun and Aubertin (1966) reported a thick exine on sterile pollen grains of ***Solanum.*** Alam and Sandal (1967) reported a persistent tapetum in male sterile sudangrass while Raj (1968) found a periplasmodium in the same species. Periplasmodial formation in chemically induced male sterile plants was reported by Dubey and Singh (1968) in Coriander.

Izhar and Frankel (1971) supposed that the faulty timing of callase activity was the cause of sterility in ***Petunia.*** Studies on amino acids

showed a decreased or lack of proline in several male-sterile plants. Increase in glycine in *sorghum*; amides in wheat; asparagine, glutamine and alanine in many other male-sterile plants are supposed to cause sterility.

Much information is now available on genes causing male sterility in the Leguminosae (Gottschalk and Kaul, 1974). Generally, these genes cause microsporogenesis breakdown at a stage specific for the gene in a species, single gene recessive and multiple gene recessive MS have been reported in this family.

In *Pisum sativum,* complete failure of cytokinesis occurred in all MMCs of the MS plants homozygous for *Ms* gene (Gottschalk and Kaul, 1974).

In soybean (*Glycine max* L.) the homozygous *ms,* plants are morphologically normal but produce abnormal pollen grains (Brim and Young, 1971). This was due to failure of cytokinesis after Telophase II of meiosis (Patil and Singh 1976).

Chauhan (1976) worked on histochemical aspects of male fertile and male sterile anthers of some flowering plants and reported that male-sterile anthers showed lesser amounts of metabolites than male fertile anthers.

Four independent recessive mutations for MS were identified in Soybean by Palmer *et al.* (1978). In all these cases MS resulted from failure of cytokinesis in meiosis.

Albertsen and Palmer (1979) reported a comparative account of microsporogenesis in MF and MS Soybeans and observed the formation of coenocytic microspores after the failure of cytokinesis after Telophase II. They concluded that the nuclear and cytoplasmic events must occur at precise times during microsporogenesis for the successful development of haploid pollen grains from diploid MMCs.

Chauhan (1979) reported that in genic male sterile ***Cucurbita maxima*** the tapetal cells become hypertrophied at the sporogenous cell stage. 'He' invaginated into the microsporangium and crushed the MMCs that resulting in MS.

In tomato (***Lycopersicon esculentum***), Sawhney and Bhadula (1988) described the morphogenesis of a stamenless-2, a single recessive gene mutant, which in homozygous conditions produce stamens that were abnormal and sterile. The mutant anthers produced microspores which later degenerate and are non-viable.

Sawhney ***et al.*** (1989) reported that in the genic MS tomato mutant sl-2/sl-2, the expression of male sterility is influenced by temperature conditions. In this mutant, gibberellins had important role in stamen development and the mutant allele seemed to affect either the biosynthesis of gibberellins or its metabolism or both, which in turn affect pollen development.

GENETICS OF MS IN *Brassica*

3. The Genetics of Male sterility in *Brassica*

3.1. Male Sterility in *Brassica*

Male sterility (MS) in *B. campestris* L. var Toria reported by the author (Zuberi *et al.*, 1981) and monogenic inheritance of the MS was determined (Zuberi and Zuberi, 1983). The present study of comparative microsporogenesis in MF and MS, plants came from populations which were of different origin. So, along with the anatomical study of anther development, an examination of the mode of inheritance of the MS was also undertaken in different cultivars or ecotypes of Toria and Yellow Sarson.

The MS lines were obtained from local cultivars of *Brassica campestris* L. Var. Toria and a released HYV of *Brassica campestris* L. Var. Yellow Sarson by self pollination.

The mode of inheritance of MS was ascertained by genetic analysis i.e. segregation of heterozygotes and test cross progenies accompanied by embryological study.

3.2. Inheritance of male sterility in *Brassica campestris* L. Var. Toria.

3.2.1. Introduction

The author obtained male sterile (MS) plants in bud selfed progeny of *B. campestris* L. var Toria during the course of a study of self incompatibility in 1978-79 in the University College London.

A total of 24 male sterile plants were obtained along with other abnormal mutants (Table I of Zuberi *et al.*, 1981). The inheritance of male sterility derived from one of these plants (1/79) was worked out by the author and was reported earlier in Zuberi and Zuberi (1983). Unfortunately seed of these male-sterile stocks lose their viability and can not be used.

When the present study was initiated during 1986-87, seven *B. campestris* L. var Toria plants were bud selfed. The seeds were obtained from the Genetics Laboratory of Botany Department, Rajshahi University and originated from local field collection in 1985. The selfed seeds were sown in fifteen pots in 1987-88 winter. Results of five bud selfed families are given in Table 3.1.

Table 3.1 Results of MF plants bud-selfed in *B. campestris* Var. Toria.

Details of plants used	No. of flowers	No. of seeds	Progeny obtained during next year	
			MF	MS
1. Plant BC-T52/88/s				
Open pollinated	9	97	51	0
Self pollinated	9	34	17	0
2. Plant BC-T54/88/s				
Open pollinated	9	87	43	0
Self pollinated	9	61	55	0
3. Plant BC-T59/88/s				
Open pollinated	9	82	32	0
Self pollinated	9	41	36	7
4. Plant BC-T54/88/s				
Open pollinated	9	73	15	0
Self pollinated	9	51	32	0
5. Plant BC-T61/88/s				
Self pollinated	8	47	28	0

3.2.2. Results

A. MS plant (MS 19-89/BC-T59-88-S/) sib mated with MF plant (MF 3/BC-T59-88-S/)

During 1987-88 five flowers from the MS plant (MS 19/ BC-T59-88-S/) were pollinated with pollen from a MF plant belonging to the same family (MF3/BC-T59-88-S/). A total of 49 seeds were obtained which were sown during the winter of 1988-89. There were 27 plants which flowered, 17 of which were MF and 10 MS. The observed results were fitted to 1:1 ratio considering the MS parent as homozygous *msms* and the MF pollen parent as heterozygous *Msms*.

Test of significance for 1 : 1 ratio.

Class	Observed	Expected	0-E	$(0-E)^2$	$(0-E)^2/E$	χ^2	P
Male fertile	17	13.5	3.5	12.25	0.91	1.82	0.20
Male sterile	10	13.5	-3.5	12.25	0.91		

The observed value of Chi square (=1.82) with one degree of freedom has a probability of 0.20 which is non-significant. Out of five selfed families only one (BC-T59/88-S/) produced seven male sterile

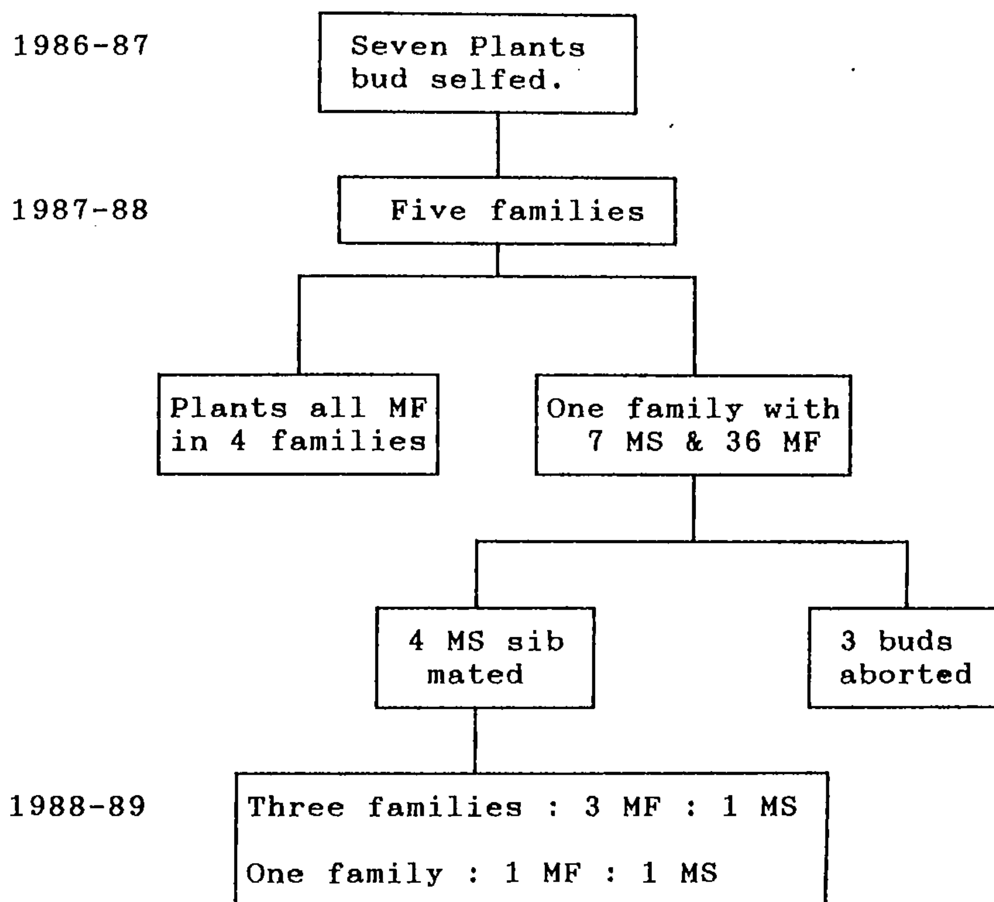
plants of which four (Table 3.2) produced enough seeds upon sib-mating with a common male parent. The anthers of the MS plants were thin and whitish in colour and were found to contain no pollen grains when collected from open MS flowers, squashed and observed under microscope. Further selfing, crossing (sib-mating) were restricted within the progeny of these four plants. Materials for anatomical and cytological studies were also collected from these families (Fig 3.1).

To confirm that the male-sterility studied in this case is controlled by a recessive gene, two types of pollinations were made. Firstly, male fertile plants of the families were selfed and the segregation for male sterility was followed. Secondly, male sterile plants were sib-mated with male fertile counterparts and segregation for male sterility was noted.

Table 3.2 Details of the Four MS plants used in raising self and sib-families in 1988-89.

MS Plants	Days to flo-wer	Plant hei-ght (cm)	No. of bra-nch	No. of frui-ts	No. of seeds/ fruit	Seed co-lour	Days to har-vest
Plant MS19-89/BC-T59-88-S/	29	70	3	51	6.5	brown	51
Plant MS20-89/BC-T59-88-S/	30	31	0	9	3.5	brown	59
Plant MS21-89/BC-T59-88-S/	31	71	0	30	5.8	brown	62
Plant MS-23-89/BC-T59-88-S/	31	68	0	17	3.1	brown	64

Fig. 3.D. Details of plants derived (*Toria*)



B. MF plant (MF3/BC-T59-88S/) bud-selfed

The MF plant (MF3/BC-T59-88S/) was bud-selfed during 1987-88. From ten bud-selfed flowers only 19 healthy seeds were obtained. When, these were sown in 1988-89, 15 plants managed to flower. The plants were very weak and produced small number of flowers. Of the 15 plants, 14 were MF and only one MS. A chi-square test was fitted for 3 : 1 ratio supposing that the MF parent was heterozygous for the gene (*Msms*).

Test of significant for 3 : 1 ratio.

Class	Obser-ved	Expec-ted	O-E	$(O-E)^2$	$(O-E)^2/E$	χ^2	P
Male Fertile	14	11.25	2.75	7.56	0.67	2.69	0.10
Male sterile	1	3.75	-2.75	7.56	2.02		

A non-significant deviation from expected 3:1 ratio indicated that the observed numbers of MF and MS follow monogenic segregation.

C. MF plant of Family (MF-31/BC-T59-88S/) bud selfed.

The seeds of 10 flowers bud-selfed on another MF plant (MF-31/BC-T59-88S/) gave 53 seeds in 1987-88 season. The seeds were sown

and 49 plants were obtained which flowered. Of these 45 were scored for MS of which 37 were MF and 8 were MS. A chi-square test was used to test the goodness of fit for 3:1 ratio.

Test of significant for 3:1 ratio.

Class	Obser-ved	Expec-ted	O-E	$(O-E)^2$	$(O-E)^2/E$	χ^2	P
Male fertile	37	33.75	3.25	10.56	0.31		
						1.25	0.20
Male sterile	8	11.25	-3.25	10.56	0.94		

The deviation was non-significant and this was a case of heterozygote *Msms* selfed to give 3:1 segregation.

D. MF plant (MF-24/BC-T59-88S) sib-mated :

The MF plant (MF-24/BC-T59-88S) sibmated to a MF pollen parent (MF-11/BC-T59-88S/).

Five flowers of the MF plant (MF-24/BC-59-88S) were pollinated with pollen from MF11/BC.59-88S/ and 72 seeds were obtained. Fifty of these were sown in 1988-89 and 47 plants were scored for MS. A total 38 MF and 9 MS plants were obtained.

A chi-square test indicated a good fit to 3:1 ratio.

Test of significance for 3:1 ratio.

Class	Obser-ved	Expec-ted	O-E	$(O-E)^2$	$(O-E)^2/E$	χ^2	P
Male fertile	38	35.25	2.75	7.56	0.21		
						0.85	0.50
Male sterile	9	11.75	-2.75	7.56	0.64		

3.2.3. Discussion

. The above four families and a large number of families like these (not reported here) all indicated that the Male sterility observed in *B. campestris* L. Var. Toria in the present case is controlled by a single Mendelian gene. The recessive allele *ms* when homozygous results in MS.

However, in all these families a deficiency in the frequency of male sterile plants was noted. A total of 106 MF and 28 MS plants were obtained, the expected numbers of MF and MS plants following Mendelian ratios would be 93.75 : 40.25. This may indicate a reduced viability of the MS plants which may be an effect of the *ms* gene. The morphological and yield characters of MF and MS plants were compared to substantiate this hypothesis (Section 3.4)

3.3. Inheritance of male sterility in *Brassica Campestris* L. Var. Yellow Sarson

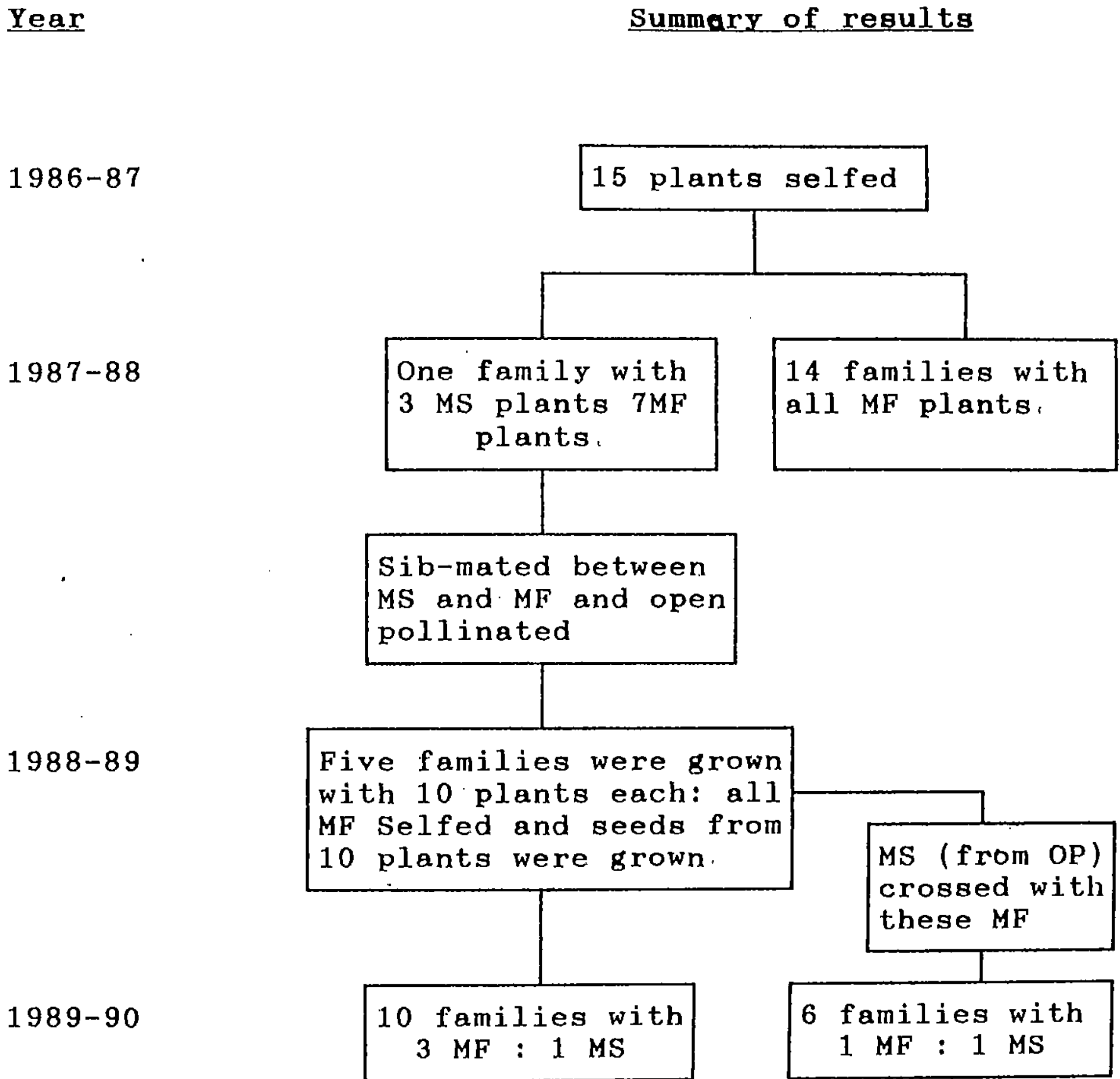
3.3.1. Introduction

In the previous section the mode of inheritance of MS in *B. campestris* var Toria was reported. During the winter of 1986-87, a high yielding variety, SS75, was grown in the field of Botany Department. The seeds were originally obtained from the Bangladesh Agricultural Research Institute (BARI), Research Station, Rajshahi. Fifteen healthy plants were selfed (Fig 3.3). It may be noted that though Yellow Sarson is self-compatible a good amount of out crossing often takes place (Chowdhury and Das, 1966). So, spontaneously appeared *ms* mutants could be expected floating in populations of Yellow Sarson masked by hetero-zygosity. In fact a few MS plants were detected by Chowdhury and Das (1966) in commercial plantations of Yellow Sarson.

3.3.2. Material and Methods

Seeds obtained from these self-pollinations were planted in the winter of 1987-88 in 15 plots. Only one family (BC-YS-21) out of the 15 was observed to contain three plants with smaller flowers possessing anthers which were narrow thin, needle-like and white in colour. Upon

Fig. 3.3. History of MS plants in Yellow Sarson



microscopic examination of these anthers, no pollen grains were observed and were identified as MS.

Crosses between MS and MF plants of the Family BC-YS21 were made in 1987-88 on the three MS plants (BC-YS21/88-MS1/, BC-YS21/88-MS2/ and BC-YS21/88-MS3). Some flowers were also left to be open-pollinated and the seeds were collected.

In all the five F_1 families grown in 1988-89 from crosses between MS and MF sibs, only MF plants were obtained (Table 3.3). The families raised from open-pollinated seeds of the MS plants were also MF. This indicated the recessivity of MS. Ten F_2 families were raised in 1989-90 from selfed seeds of 10 MF plants belonging to the five F_1 families (Table 3.3).

3.3.3. Results

The results are summarised in Table 3.3. All the families showed a 3 : 1 segregation for MF and MS plants.

When seven families with MS plants originating from open pollinated progenies of the MS plants were grown, all of them exhibited similar segregation of 3 : 1 for MF and MS plants.

Table 3.3 Segregation in F_2 generation of crosses between MS and MF plants of three families from BC-YS 21

Crosses made 1987-88	F_1 in 1988-89	F_2 in 1989-90	Number of plants MF	Number of plants MS	Chi square (3:1)	P
BC-YS21/88MS$_1$	10 plants	3 Fami-	22	6	0.19	0.50
x BC-YS21/88MF$_5$	all MF	lies	7	1	0.67	0.30
			20	8	0.19	0.70
BC-YS21/88 MS$_2$/	15 plants	4 Fami-	31	8	0.417	0.50
x BC-YS21/88MF$_8$	all MF	lies	11	3	0.095	0.70
			25	8	0.009	0.90
			33	10	0.069	0.80
BC-YS21/88 MS$_3$/	10 plants	3 fami-	35	12	0.006	0.90
x BC-YS21/88MF$_9$	all MF	lies	13	4	0.019	0.90
			30	11	0.072	0.70
	Total		227	71		

. Data on segregation in the progeny of MS pollinated with F_1 MF plants in 1988-89 are given in Table 3.4. As expected, all the six families from crosses between MS and MF heterozygous plants have shown a ratio of I MF and I MS plants.

3.3.4. Discussion.

All these data reveal monogenic control of MS in Yellow Sarson, the recessive allele *ms* when present in homozygous condition resulted in complete MS in Yellow Sarson too. The observed numbers of MS plants were always fewer than expected in the families studied (observed 227 MF and 71 MS) but expected following Mendelian ratios (223.5 MF and 74.5 MS). A lower fertility or survival of the MS plants was indicated (Section 3.4).

The allelic relationship between the *ms* gene in Toria and in Yellow Sarson could not be determined due to lack of time. This can be done by crossing the heterozygotes of one with the MS plant of the other. If the genes are allelic, this would give 1:1 segregation of MF and MS (a back cross). But the presence of non-allelic genes would be indicated by the presence of all MF in the family. Chowdhury and Das (1967) observed non-allelic genes controlling MS in SI and SC cultivars of Brown Sarson.

Table 3.4 . Segregation of test crosses made during 1988-89 between MS and F_1 MF plants

Test crosses	Test cross progeny in 1989-90		χ_2 1 : 1	P
	MF	MS		
	25	21	0.35	0.80
	10	14	0.67	0.30
BC-YS21/88 MS_1op/MS_3	15	14	0.03	0.80
Y BC-YS21/88F_1/89MF_5	25	20	0.35	0.80
	29	24	0.47	0.30
	32	25	0.43	0.50
	136	118	1.28	0.20

3.4. Differences between MS and MF plants for morphological and reproductive characters

3.4.1. Introduction

The successful use of male sterility in hybrid seed production depends upon the reproductive performance of the male sterile genotypes. As the material used in the present study was originally obtained form self-pollination and successive generations of sib-mating, the MS and MF lines are subjected to fairly strong inbreeding. This may result in inbreeding depression and decrease in vigour of these lines. Moreover, in all families examined in Section 3.3, a fewer than expected MS plants were observed. It is important to compare the performance of MS and MF lines in respect to important morphological and reproductive characters.

3.4.2. Materials and Methods

The sib-mated Family MS-21/BC-T59-889 grown in 1 x 2 m plots in the field of Botany Department during the winter of 1988-89. The families were assigned randomly to the plots in rows with 30 cm space between rows and 10 cm space between plants. The following morphological and reproductive characters were measured on five randomly chosen plants of each of MS and MF phenotypes:

flower diameter (mm), petal length (mm), gynoecium length (mm), plant height (cm), branch number, leaf number, fruit number, flower number, number of seeds per fruit and seed yield per plant.

Flower characters were scored from the random samples during flowering and the other traits were scored when plants were matured.

3.4.3. Results

It is noted that the MS plants flowered later than the MF counter parts (Table 3.5.) indicating slower growth. The data on the morphological characters were given in the Table along with the result of 't' test. The flowers of MS plants were significantly smaller than the MF flowers as revealed from flower diameter and petal length (**Fig. 3.1 and 3.2**). The gynoecium length was, however, not significantly different in MS and MF plants. The MS plants were also found to be significantly smaller than the MF plants; the plant height, number of branches, and number of leaves were smaller in MS plants.

For yield contributing characters, i.e., number of flowers and fruits, number of seeds per fruit and seed yield per plant, the MS plants showed inferior performance than the MF plants. However, the differences for number of seeds per fruit and seed yield per plant were not statistically significant.

Fig 3.1 Male Fertile (MF) and Male sterile (MS)
Flowers of <u>Brassica</u> <u>campestris</u> . Var Toria

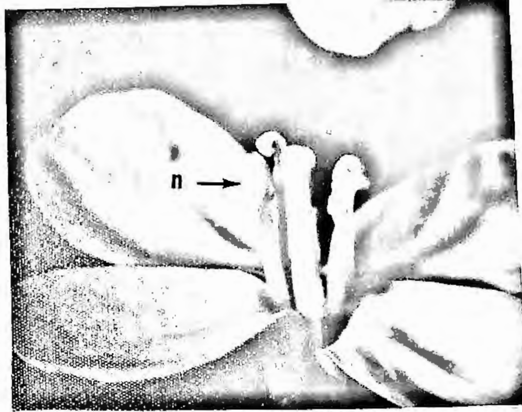
MF

MS

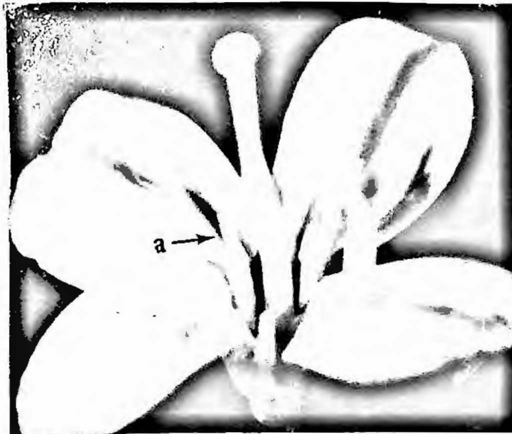
MS MS MF

Fig 3.1

Fig 3.2 Closer view of MF and MS flowers of
Brassica campestris L var *Yellow Sarson*



MF



MS

Fig 3.2

Table 3.5 Comparison between MS and MF plants for morphological characters

		MS	MF	t	P
Plant height (cm):	$\bar{X}$ =	60.9	75.4	3.20	**
	S =	6.5	7.1		
Branch No.	$\bar{X}$ =	2.95	9.41	4.04	**
	S =	1.34	3.51		
Leaf No.	$\bar{X}$ =	9.18	11.97	3.91	**
	S =	0.71	1.43		
Fruit No.	$\bar{X}$ =	11.91	19.26	2.98	*
	S =	4.11	3.31		
Flower No.	$\bar{X}$ =	18.89	27.11	2.44	*
	S =	5.15	4.72		
Seed No./fruit	$\bar{X}$ =	9.51	11.46	1.09	NS
	S =	3.21	2.15		
Seed yield/plant(g)	$\bar{X}$ =	1.89	2.98	1.04	NS
	S =	1.25	1.91		
Flower diameter	$\bar{X}$ =	11.20	13.20	3.78	**
	S =	1.15	0.27		
Petal length	$\bar{X}$ =	8.2	9.70	4.17	**
	S =	0.76	0.27		
Gynoecium length	$\bar{X}$ =	6.20	6.40	0.89	NS
	S =	0.96	0.76		
Flowering time	$\bar{X}$ =	33.7	31.00	1.01	NS
	S =	1.6	1.1		

3.4.4. Discussion

The results indicated a reduced vigour of MS plants. Chowdhury and Das (1967a) reported that MS plants of ***B. campestris*** Var. Yellow Sarson resembled normal plants in their morphological characters, vegetative growth, flowering time and maturity period but the flowers and particularly petals of MS plants were small. The differences between MS and MF plants of same family were highly significant for petal length, length of blade, width of blade and anther length (Chowdhury and Das, 1967b). The reduced flower size was again observed in case of MS plants of ***B. campestris*** L. Var. Toria in the present study.

The reduced flower size may be due to the pleiotropic effect of the ***ms*** gene. However, gynoecium length and number of seeds per fruit were not affected significantly. This suggests that the pleiotropy affects the petals only. Normal seed production demonstrates that the female fertility of the MS plant was not affected by the ***ms*** gene.

As a consequence of the smaller size in MS plant, these MS flowers may have a smaller number of insect visitors resulting in reduced seed set. Number of seed per fruit and seed yield per plant indicate such a trend.

A general reduction in flower size in the case of MS plants has also been reported in flax (Bateson and Gairdner, 1921), maize (Beadle, 1932), tomato (Rick, 1944; Rick and Robinson 1951; and Bishop, 1954); Onion (Peterson and Foskett, 1953), Cowpea (Sen and Bhowal, 1962). This

indicates that *ms* gene is really affecting flower size in a wide variety of plant species.

Utilization of male sterility in hybrid seed production programs has some general problems such as identification of MS plants, maintenance of good inbred lines, transferring of male sterility to other lines, production of MS plants etc. In the present case, the genetic male sterility also has some difficulties of maintenance of the *ms* gene and production of MS plants.

Morphological differences in vegetative and floral characters between MS and MF plants is of considerable interest to the plant breeders because this can help in the easy recognition. The maintenance and continued production of MS plants will involve the production of families (*msms* x *Msms*), progeny of which will give 1 : 1 segregation and half of the plants (which are MF) has to be rouged out and other half of MS plants will produce hybrid seeds. Rouging out of MF plants in the hybrid seed production plots will be easier as the MS plants can be identified by the small flower size and shrunken and white anthers as they start flowering.

The reduced performance of the MS plants for morphological and reproductive characters and the fewer MS plants in Families studied indicate lowered fitness of *ms* gene or closely linked semi-lethal gene (see Zuberi *et al.,* 1981). Further study on these line may provide interesting results.

MICROSPOROGENESIS

4. Comparative study of microsporogenesis in MF and MS flowers.

4.1. Introduction

The discovery of male sterility (MS) provided the plant breeders a suitable tool for the production of hybrid seeds (Chowdhury and Varghese, 1968; Edwardson 1970; Heslop-Harison, 1972). However, the mechanism or mechanisms responsible for Male sterility are still not clearly understood in many cases. Microscopic, histological and biochemical methods frequently have been used to determine the differences between MF and MS lines. So far, no precise and consistent pattern has been noted among various taxa displaying MS, suggesting that more than one mechanism might be involved and that the time of abortion differs among the taxa.

Comparative studies of microsporogenesis in both MF and MS lines have been made by Artschwager (1947) in sugar-beet , Singh and Hadley (1961) in squash and Zenkteler (1962) in carrots, to learn how male sterility is caused.

These studies have revealed that in cytoplasmic MS e.g. sugar-beet , sorghum and carrot, pollen abortion is associated with abnormal behavior of the tapetum. In squash where sterility is of a genetic type, absence of meiosis in the pollen mother cells (PMC) and subsequent

degeneration of the mother cells leads to sterility.

Erichsen and Ross (1963) have reported a delayed degeneration of tapetal cell in colchicine-induced male sterile plants of sorghum. Dubey and Singh (1965) reported failure of the tapetal cells to degenerate at a proper stage as the cause of MS in flax.

In recent years, the comparative studies of morphogenesis in MF and MS lines, the following aspects were looked into a) when exactly the abortion of sporogenous cells occur; b) what happens when the abortion begins, c) what clues can be observed before the onset of abortion and d) what related observable events occur in the tapetum and microspores during the stages leading to abortion..

Microsporogenesis can be defined as "......including all stages from the undifferentiated microsporangium to the mature pollen-filled anther including related events in the tapetum and external wall layers. ...." (Horner and Lersten, 1971). In rapeseed no detailed account of microsporogenesis has been reported. No comparative developmental study of microsporogenesis in MF and MS rapeseed is available. To understand the process of male sterility, such accounts are essential.

Therefore, a comparative study of microsporogenesis and development of anthers in MF and MS lines of rapeseed was undertaken in order to determine the mechanism of male sterility.

4.2. Materials and Methods

4.2.1. Materials

Several independent families of *B. campestris* L. were raised as described in Section 3 for the study of inheritance of MS. Reproductive buds of various stages of development were collected form MF and MS plants of two Families BC-T59-88S/19-31 and BC-YS-21-88/1-51 for anatomical examination to study microsporogenesis. Two other families of *B. campestris* L. Var. Toria maintained in the Population Genetics Laboratory of Botany Department were observed to contain MS plants when selfed. The population BH-86 was established from collection from Mohishmary village of Natore District and was maintained as an open-pollinated population. In 1988-89 five plants were bud-selfed and one Family BC-T-BH86/59/31-S/ from these were found to contain 17 MF and 10 MS plants in 1989-90. Flower buds from these MS and MF plants were used (Section 6.3). Another population, K-86, originated from a local collection in 1986 and maintained by open pollination was used as a source, seven plants were bud-selfed in 1988-89 and one Family originated from these (BC-T-K86/19/3-S) produced 5 MF and 2 MS plants in 1989-90. These were the source of floral-buds for the study of microsporogenesis reported in Section 6.4.

4.2.3. Collection of flower buds

The developing buds were collected during the period December to January, from plants grown either in earthenware pots or in the experimental fields.

In *Brassica campestris* L. like other members of the Cruciferae, anthesis takes place from the lower to the upper part of the inflorescence. A receme at antheis, therefore, contains flowers at 'open stage' at the lower and flower buds of various stages of development at the upper part. By taking the point between the largest bud and the most recently opened flower as O, each bud can be designated by its position as -1, -2, -3, -4 and so on and each open flower as +1, +2, +3 (Fig 4.1).

The flower buds for the study of development of anthers and microsporogenesis were usually collected from inflorescence with 5 to 10 open flowers. It was observed that buds collected from more advanced inflorescence into flowering (with many developing fruits and open flowers) do not give good sections and right anthers.

It was reported that flowers in the lower portion of the inflorescence have maximum number of pollen grains and the number of pollen grains in the upper end gradually tend to have fewer number of pollen grains than basal flowers (Hinata and Konno, 1975). In fact, they reported that the number of pollen grains per anther were reduced by half at the terminal part of the inflorescence. Moreover, in most cases

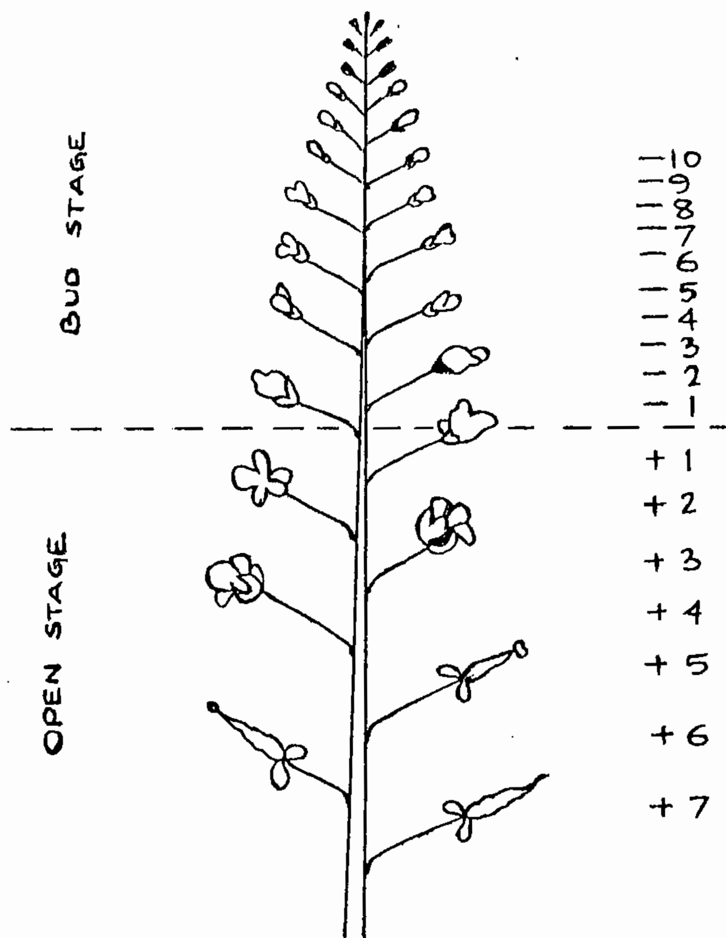


Fig 4.1 Diagrammatic representation of the inflorescence of <u>B.campestris</u>

the main inflorescence was used for the study which contains healthy buds whereas inflorescence developed on lateral branches tend to bear fewer, smaller buds with weak anthers.

Records were kept as to the position of the sampled buds on the inflorescence while collecting them for fixation and embedding. Experience showed that flower buds of -1 to -7 stages would have anthers where meiosis was completed and these anthers contain locules with mature pollen grains. Younger buds of -12 to -8 stages of floral development usually have anthers with microspores and still younger anthers with MMC's in very early stages of development. The speed of development however, depend on the general health and vigour of the plant, temperature, availability of water and on the time of the season when the flower is opening.

4.2.4. Fixation, Embedding, Sectioning and Staining

The process involves fixing the plant material in a fixative which is followed by washing, dehydrating, infiltrating, embedding in paraffin and sectioning with the aid of a microtome. The paraffin sections are then stained with Sharman's Orange G-Tannic Acid stain.

1. The plant material was fixed in a fixative (FAA) in a screw cap vial and the particulars of the material, name of the fixative and date of fixation were written down on a white tag.

2. Air was evacuated from the materials in the fixative with the help of a suction pump to facilitate the entry of the fixative in the material properly.

3. The specimen was allowed (with fixative) to stay in the bottle at least for 24 hours and then was transferred to fresh fixative.

Dehydration

The removal of water from the plant tissues is called dehydration. Dehydration was done gradually in the following ways keeping the materials for 24 hour in each step.

Fixative→50%, → 60%, → 70%, → 80%, → 90% → and 100% alchohol (2 hours)

100% alchohol → absolute alchohol + chloroform (2:1)

→ absolute alchohol + chloroform (1:1), Erytrosin B added to the mixture for temporary staining the material,

→ Absolute alchohol+Chloroform (1:2) → Pure chloroform (2 changes)

Infiltration in paraffin

The plant material which has been dehydrated and cleared in pure clearing reagent i.e., chloroform was infiltrated in paraffin wax.

A separate clean vial was taken and molten paraffin (58°C-60°C MP) was poured in the vial from the stock molten paraffin which is kept in a beaker in an incubator running at 65°C. The molten paraffin was allowed to solidify in air. The material along with the chloroform was poured on the top of the solidified paraffin layer in the bottle. The label was transferred from the original vial to the paraffin vial.The vial with paraffin, material and chloroform was transferred immediately to the incubator which is running at 65°C. The vial was kept there for 24 hours.

The solution of paraffin and chloroform from the vial was poured off and fresh molten paraffin was added to the vial and was kept in the incubator. Two changes of fresh paraffin was given to remove the last traces of chloroform. The vial with the material and the paraffin was kept in the incubator for 5 days for thorough infiltration of paraffin in the material.

Embedding

Embedding is the next step after the infiltration is completed. It involves pouring of molten paraffin and the material into suitable receptacle, arranging the materials in proper order and then cooling the entire mass quickly in cold water to get solidified within the receptacle.

Preparation of paraffin Block

A spirit lamp was kept nearby for heating the needle which was used for arranging the materials in the molten paraffin inside the tray. A vessel containing cold water was kept nearby for cooling the paraffin block quickly. The specimen tube containing materials in the molten paraffin was taken out from the incubator. The specimen tube was shaked well to get the materials off from the bottle and was quickly poured along with the materials into the tray. Some more molten wax from the stock was added quickly to the tray to cover the material properly. The tag was transferred from the tube to one side of the tray. The needle was heated in the flame and the materials were arranged quickly in the molten wax in the tray. Then the tray was transferred carefully to the vessel of cold water without disturbing the arrangement of the materials and allowed to float for some time. The tray was immersed carefully beneath the surface of the water and a small weight was put over it.

The tray was allowed to remain in water for 24 hours so that the paraffin is fully cooled and solidified. The tray was then taken out from water and was allowed to dry in air for 24 hours. When the tray was completely dried, the paraffin block was taken out by unfolding the tray.

Microtoming

A small block of wax containing one material was cut and removed from the entire paraffin block with the help of a sharp scalpel. The block was trimmed so that the object was exactly in the center of perfectly trimmed rectangular piece of wax. The axis of the object was exactly parallel to the long side of the block. The block to be sectioned was mounted onto the metal holder by melting the paraffin coating of the holder with a hot spatula. The block with the holder was immersed in ice-water and then kept in a refrigerator.

For section cutting, the holder was inserted into the jaws of the microtome and the holder was secured to the microtome. The knife-holder and the screws holding it were made tight. The knife was set at 45°. The thickness was set at 10 µ. The sections were cut till the entire width of the block was cut against the edge of the microtome. The ribbons were lifted with a brush in such a manner as to allow a few sections remain lying on the surface of knife so that the ribbon did not break.

The ribbons were kept on a thick sheet of paper with glossy surface the cut prices of ribbons were mounted serially on clean slide. The slides were first smeared with Haupts adhesive evenly on the slide and were flooded with 4% percent Formalin. These pieces of ribbons were taken on the slide and were arranged serially so that they lie parallel

to each other. Care was taken that the glossy lower surface of the ribbon must face the slide.

The slides with ribbons were transferred carefully on the slide warmer which is kept at 45°C. Due to temperature the ribbons were stretched and they were straightened with the help of two blunt needles. The excess formalin was drained off with the help of blotting paper. These slides were allowed to remain on the slide warmer for at least 3-4 days.

Staining and mounting

To remove the wax before the sections are stained, the slides were dipped and allowed to stand for 4-5 minutes in a Coplin Jar containing xylene. The slides were then transferred to a 1:1 solution of Xylene and Absolute Alcohol, then to Absolute Alcohol; and then hydrated by passing it through an alcohol series of 90%, 70%, 50%, alcohol and finally into water.

The sections were stained by Orange G–Tannic acid following the schedule given below.

1. From water to an aqueous 2% zinc chloride . . . . 1 minute.
2. Rinsed in water . . . . 5 seconds.
3. Stained in 1/25,000 aqueous safranin . . . . 10 minutes.
4. Rinsed in water . . . . 5 seconds.

5. Stained in Orange G-Tannic Acid solution . . . . . . 10 minutes.

6. Rinsed quickly in water . . . . 2-3 seconds.

7. Treated in 1% aqueous Iron Alum . . . . 2 minutes.

8. Rinsed in water . . . . 15 seconds.

9. 50% Alcohol . . . . 5 seconds.

10. 70% Alcohol . . . . 5 seconds.

11. 90% Alcohol . . . . 5 seconds.

12. Absolute Alcohol . . . . 5 seconds.

13. Absolute Alcohol . . . . 5 seconds.

14. Absolute Alcohol + xylol (1:1) . . . . 5 seconds.

15. Absolute Alcohol + xylol (1:1) . . . . 3 minutes.

16. Xylol . . . . 5 minutes.

17. Xylol . . . . 2 minutes.

18. Mounted in Balsam.

Chemical reagents

Safranin 1/25,000 aqueous 4 ml, 1% aqueous safranin to 1 liter water.

Orange G-Tannic acid : Orange G 0.5 gm. Tannic acid 10 gm; Conc. HCl 4 drops, Thymol, a few crystals; water to 100 ml.

2% Zinc chloride (aqueous) : Zinc chloride 2 grms, water to 100 ml.

1% Aqueous Iron Alum (Ferric Ammonium sulphate) : 1 gm. of Iron Alum to 100 ml. water.

4.2.5 Photomicrography

The photomicrographs were taken by a Swift Master Research Microscope fitted with camera. Black and white films (No.7. ASA 100)and colour films (Fujicolour, ASA 100) were used. The photographs were taken by using 12X and 10X eye pieces and 10X, 20X, 40X objectives.

4.3. Floral Initiations

Number of days required to flower, flowering time (FT), for various families of *B. campestris* L. Var. Toria and Yellow Sarson are given in Table 4.1. Floral initiation required one to two days more in MS plants than their MF counter-parts but the differences were not significant. The flowers open three to four hours after sunrise in the morning at about 9 AM depending on the brightness of the day and remain for two to three days before the petals drop off. The flowers are bright yellow in colour and are borne on long racemes. There are six stamens in two whorls, tetradynamous in arrangement, the inner four long and the outer two short. In MS plants all six stamens become sterile. The bi-carpellary pistil is long, syncarpous, one chambered, stigma one, globose, style short, ovary long, one chambered separated into two parts by a false septum, called replum, ovules 10-20 in Toria but 25-30 in Yellow Sarson, placentation parietal.

Table 4.1 Flowering Time of MF and MS plants of families of Toria and Yellow Sarson

Family	Flowering time (days)	
	MF	MS
1. BC-T59-885/19-3	31.0	33.7
2. BC-T59-885/31S	29.1	31.9
3. BC-T-BH-86/59/31-S)	33.2	35.8
4. BC-T-K-86/9/3-S)	35.5	36.9
5. BC-YS-21-88/1-5	40.5	43.3

DEVELOPMENT OF ANTHERS IN *Brassica*

5. Development of Anthers in *Brassica*

5.1. Early development

The development of anther and microsporogenesis occur similarly in *Brassica* as in other dicot species. A study of microsporogenesis in MF rapeseed is necessary to provide comparison with the events before and after microspore breakdown in MS anthers.

The anther is tetrasporangiate (Fig. 5.1). The development of anther is usually alike in the MF and MS plants till later stages. The young anther is a four lobed structure (Fig. 5.2). The multicellular archesporium is differentiated in the hypodermal region at each of the four corners of the young anther. Later the archesporial cells undergo a periclinal division giving rise to an outer layer of primary parietal cells and an inner layer of sporogenous cells (Fig. 5.2). The primary parietal layer undergoes further divisions giving rise to wall layers. The anther wall at the microspore mother cell (MMC) stage consists of, in addition to the epidermis, an endothecial layer, two middle layers and a tapetal layer (Fig 5.3). Each lobe of the anther has a central region of primary sporogenous tissues surrounded by the wall layers (Fig. 5.3). Primary sporogenous tissues give rise to 9 to 18 columns of angular PMC's (Fig. 5.4). The ontogeny of the anther in *B. campestris* is given below :

Fig 5.1 T.S. of young bud of *Brassica campestris* L. showing early development of anther.

Fig 5.2 T.S. of young MF anther showing undifferentiated parietal (pa) and sporogenous (sp) cells with deep staining nuclei.

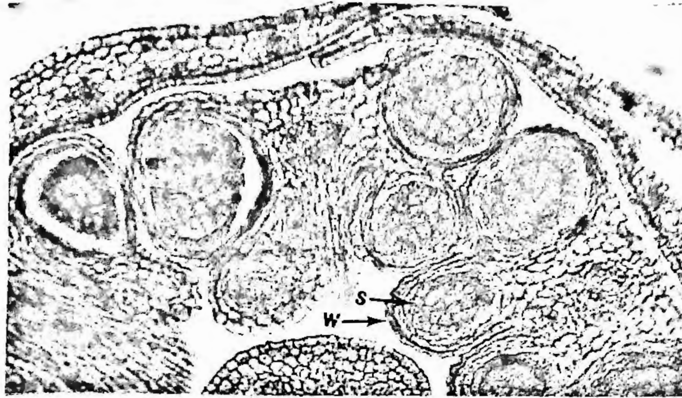


Fig 5.1

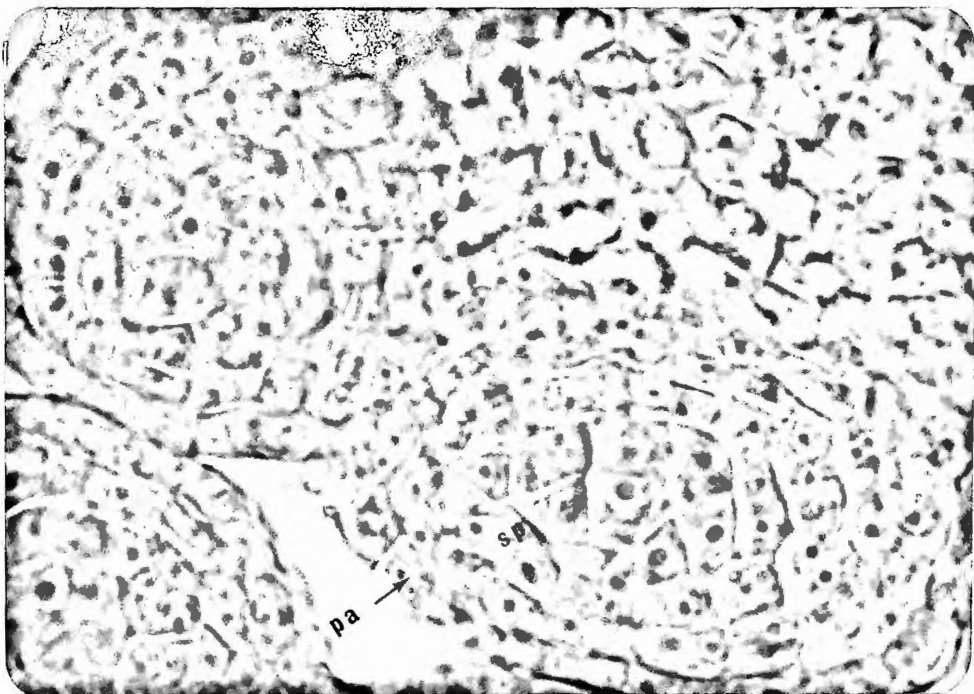


Fig 5.2

Fig 5.3 T.S. of young MF anther showing the epidermis (ep), endothecial layer(en), two middle layers (mi), a tapetal layer(ta) and the central mass of sporogenous cells (sp).

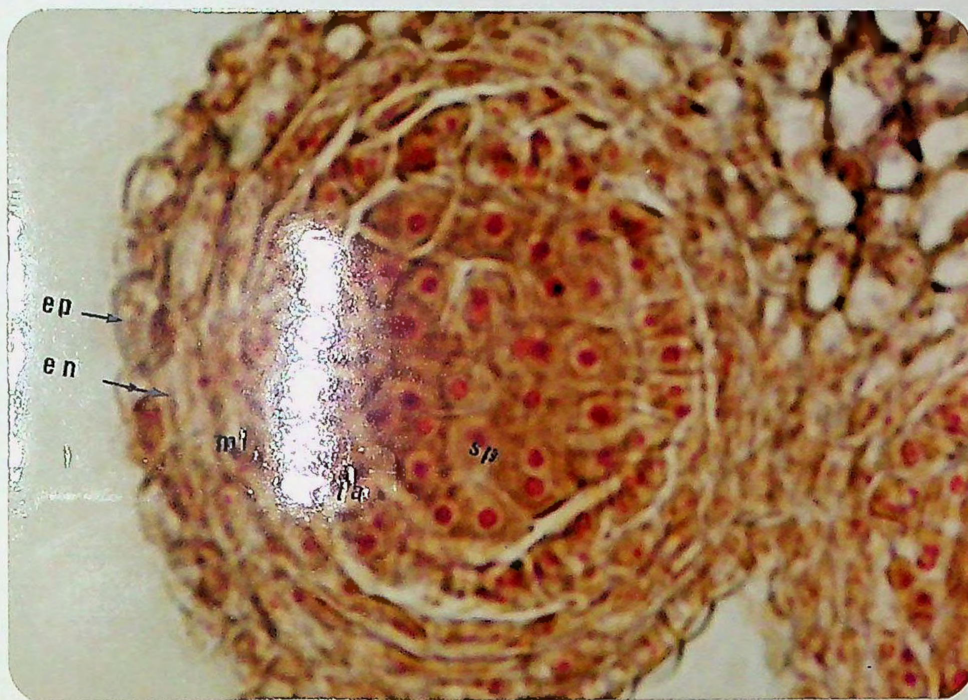


Fig 5.3

Fig 5.4 T.S. of MF anther loculus showing central mass of angular PMC (P) with large nucleus (n), single layered tapetum(T) & epidermis(E)

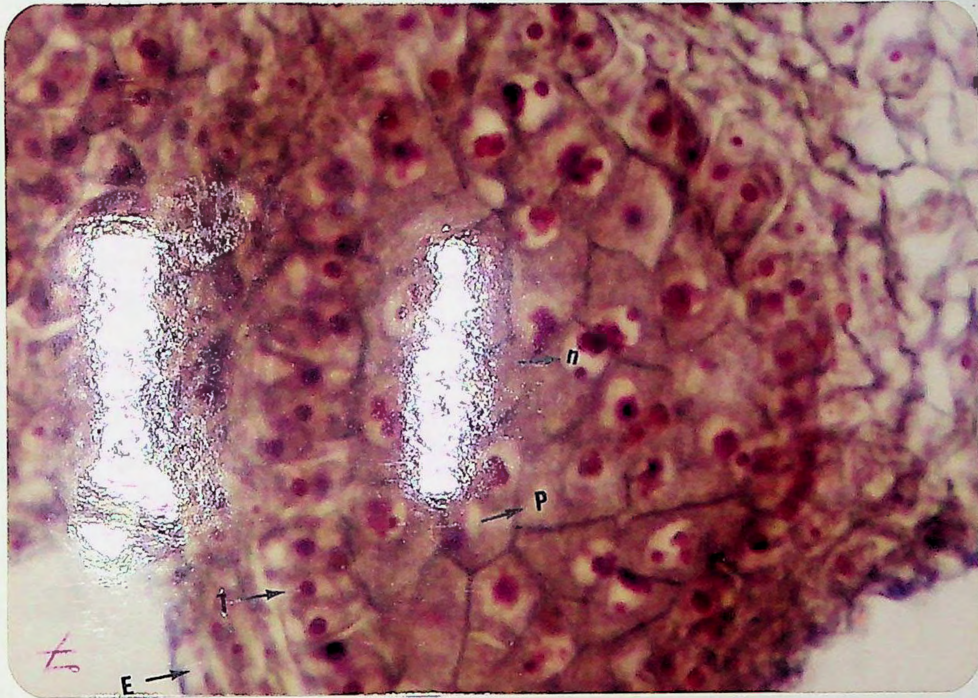


Fig 5.4

5.2. Development of Endothecium and anther wall

The microsporangium is found to develop a wall consisting of epidermis, two secondary parietal layers and a mass of sporogenous tissue. The endothecium arises by division of outer secondary parietal layer adjacent to the epidermis. The endothecium is single-layered (Fig. 5.3) and the cells remain thin-walled till the microspores differentiate and become vacuolate. By the time vacuolate pollen stage is reached, the tapetal cells breakdown and disintegrate (Fig. 5.5). Concurrently, the endothecial cells elongate radially and characteristic thickenings develop on their radial walls (Fig. 5.6). This deposition of fibrous bands in the endothecium coincides with the initiation of tapetal cell degeneration. The middle layers are ephemeral, no tapetum or middle layers are present (Fig. 5.6). They gradually flatten and become crushed at this stage. The anther wall at the time of dehiscence is represented by a very flattened epidermis and a prominent layer of endothecium (Fig. 5.6 and 5.7).

In MS plants, the development of anther wall including the endothecium follows the same pattern as in their MF counterparts upto the MMC stage. However, the behaviour changes differently in subsequent stages in various MS lines. These individual cases of MS will be described separately.

Fig 5.5 L.S. of a part of MF anther showing the degenerating tapetum (t) and mature pollen grains (P)

Fig 5.6 T.S. of a mature MF anther with radial thickenings (ra) on the endothecial cell walls (en). Pollen grains with deeply stained cytoplasm (p ;) and the thick walled epidermis (E) are shown.

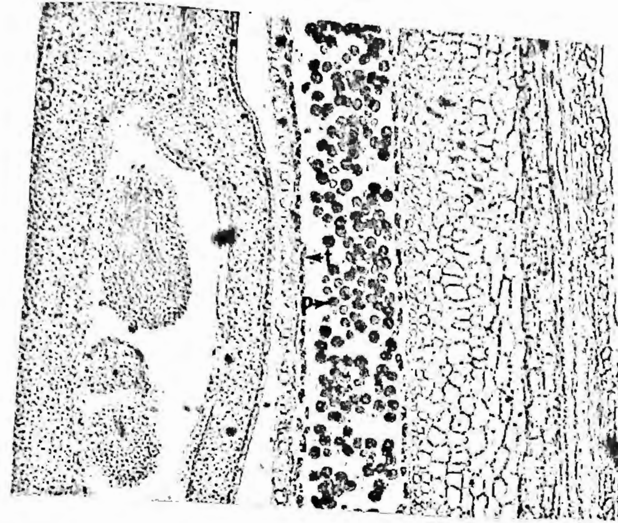


Fig 5.5

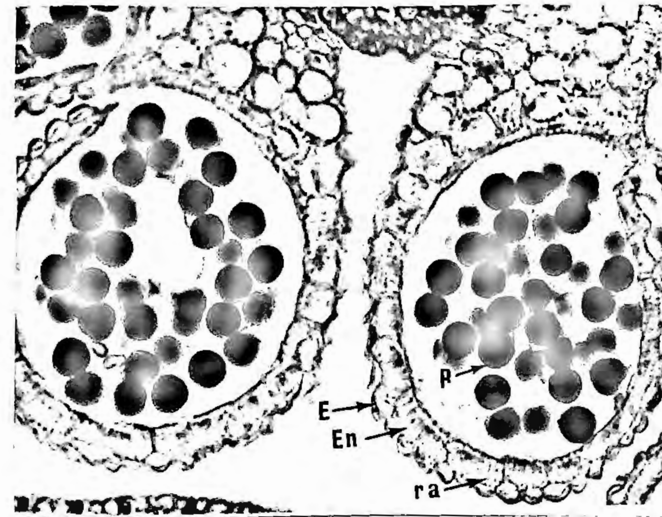


Fig 5.6

Fig 5.7a T.S of MF anther loculus with degenerating tapetum (t) and pollen grains (p)

Fig 5.7b T.S. of MF anther showing normal tapetum (t), flattened endodermis (e) and epidermis with nearly mature pollen grains (p)

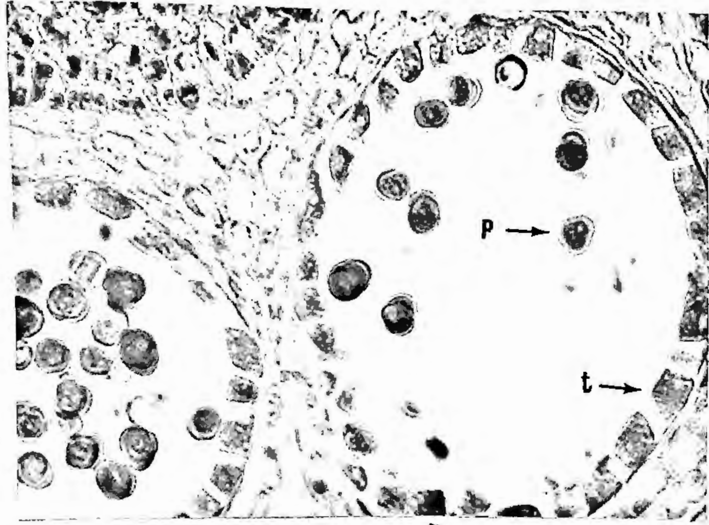


Fig 5.7 a

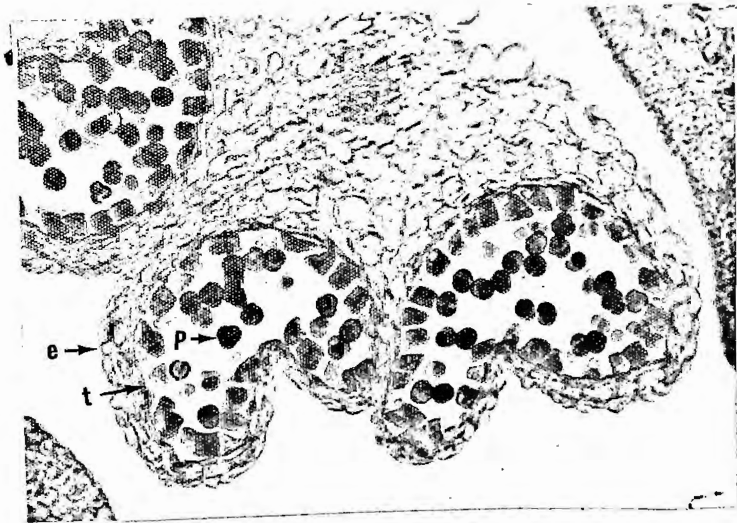


Fig 5.7 b

5.3. Behaviour of Tapetum

As indicated above, the tapetal cells originate from the small and oblong cells of inner secondary parietal layer adjacent to the sporogenous mass (Fig. 5.2). When the MMC's undergo nuclear enlargement as well as overall cellular enlargement, the tapetal cells also begin to enlarge and to stain more intensely than other anther wall layer cells (Fig. 5.3). There were large vacuoles in each of the tapetal cells. With the completion of meiotic cell division of the MMC's, the tapetum disintegrates completely in the MF plants, the anther loculi at this stage is bordered only in the epidermis and the endothecium (Fig. 5.5). Eventually the partition between neighboring locule of the same anther lobes becomes thin at the stomium and breakdown forming a large cavity, the theca (Fig. 5.6). During dehiscence, the thin row of cells connecting the cells at the stomium breaks. The pollen grains are well developed with a thick exine and deeply stained cytoplasm (Fig. 5.6).

In MS plants, the tapetum develops as in MF plants till the premeiotic stage. The fate of tapetum before, during or after meiosis depends on the type of MS as described separately in Sections 6.1, 6.2, 6.3, and 6.4.

5.4. Events in the sporogenous tissue

As the four tissues (epidermis, parietal layers, tapetum and sporogenous tissue) become distinguishable from each other early in development, the sporogenous cells form a mass of cells which were deeply stained (Fig. 5.2 and 5.3). Each sporogenous cell is surrounded by a primary wall, the densely stained cytoplasm contains many vacuoles (Fig. 5.4). As they develop from sporogenous mass to meiocytes, the angular MMC'S undergo nuclear as well as over all cellular enlargement (Fig. 5.4). Entering meiosis, each cell begins to become spherical. By the end of meiosis, the locule begins enlarging, each microspore develops its wall, separates from the tetrad, enlarges and takes the round shape of an individual microspore.

As sporogenesis proceeds, the released microspores enlarge and become more vacuolate. The exine thickens considerably around each microspore, the tapetum still persists with its dense cytoplasm.

Mitosis occurs, forming vacuolate pollen grains which accompany the degeneration of the tapetum and the thickening of the radial walls of the endothecial cells (Fig. 5.6).

In MS anthers the early developmental stages are comparable to those of the MF lines as described above. The four distinct tissues are formed with the same spatial relationship. Anatomically, both MF and MS lines are similar through the pre-meiotic stages. Succeeding events of

microsporogenesis show considerable differences between various MS types from their MF counterparts. The four types of MS with the different sporogenesis consequences are described in the sections 6.1 to 6.4.

DEVELOPMENT OF MALE STERILE ANTHERS

6. Development of Male sterile Anthers

6.1. Family BC-T59-88S/19-31

Anthers from MF and MS plants from the above family when examined for microsporangia revealed that upto premeiotic stage there was no difference between MF and MS. While the fertile anthers completing meiosis normally, in the MS anthers the first meiotic division was initiated but neither karyokinesis nor cytokinesis was completed. Instead, the meiocytes in the MS anthers became enlarged, the nuclei gradually lose stainability and large vacuolated areas appeared in the cytoplasm and close to the nucleus (Fig. 6.1). The cytoplasm of the meiocytes became granular and rapid disintegration of the microsporocytes . (Fig. 6.2).

Differences with regard to the behaviour of endothecium, epidermis and the middle layers were observed between MF and MS plants of this family. In the MF anthers, the epidermal and the endothecial cells become stretched greatly during the course of anther development and enlargement (Fig. 5.4). At maturity, stretched cells of the epidermis can be seen adhering to the outer walls of the endothecial cells which by then reach their maximum development and bear fibrous bands of thickenings on their radial walls (Fig. 5.6). In the MS anthers, however, the epidermal cells remain unstretched (Fig. 6.3). The

Fig 6.1 T.S. of young MS anther showing initial changes leading to disintegration of meiocytes(S), epidermis(E) & tapetum(T)

Fig 6.2 T.S. of MS anther showing late stage of degeneration of meiocytes(S), tapetum (T) loosing its stainability.

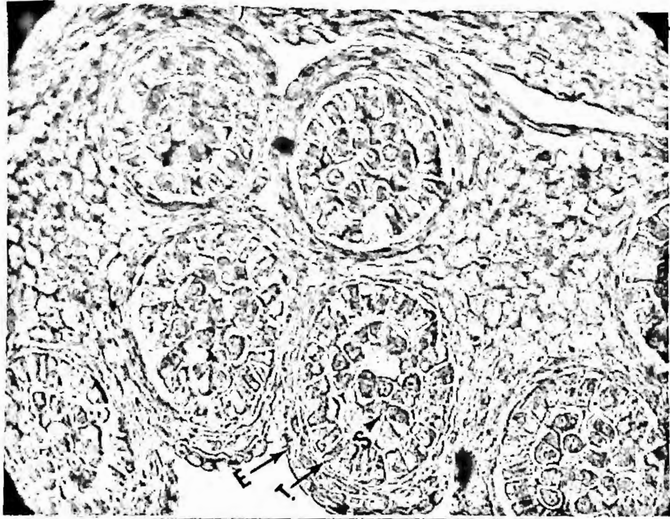


Fig 6.1

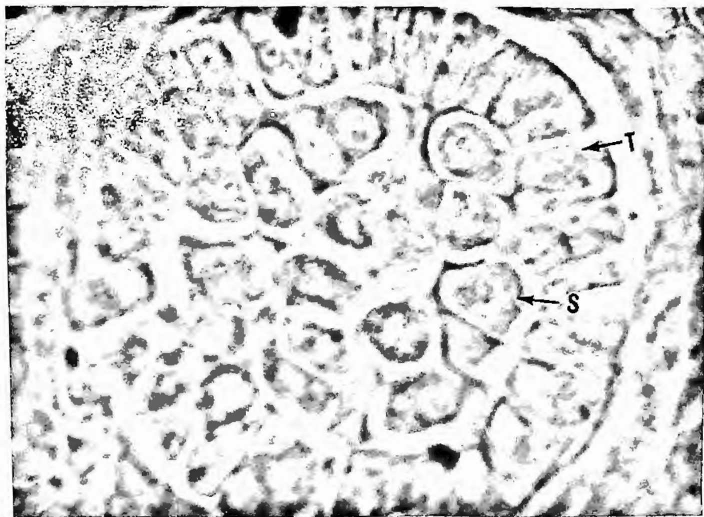


Fig 6.2

Fig 6.3 T.S. of MS anther with degenerating meiocytes (S), black cytoplasm (B), prominent tapetum (T), parietal cells (P) and epidermis (E)

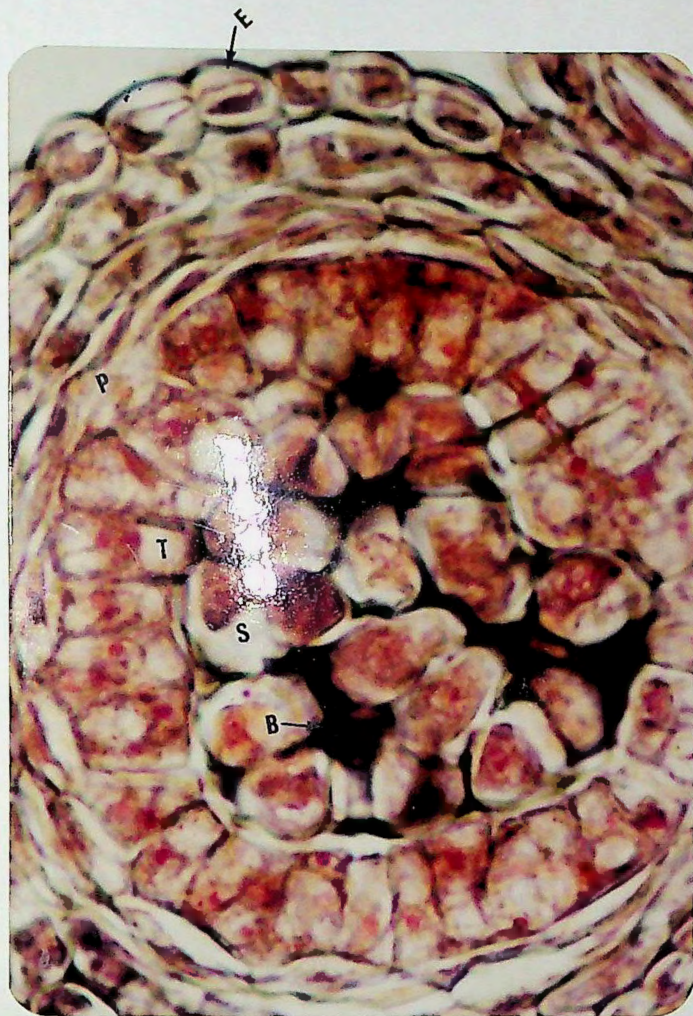


Fig 6.3

Fig 6.4 T.S. of MS anther showing degenerating meiocytes (S), tapetum (T) with epidermal (E) and endodermal (En) cells

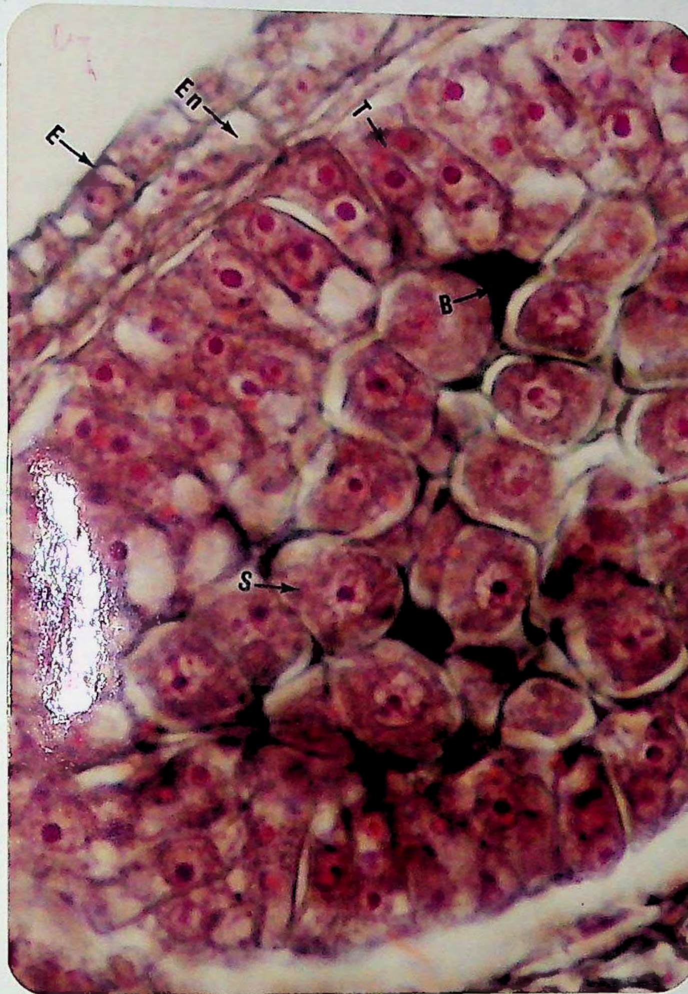


Fig 6.4

Fig 6.5 L.S. of MS anther showing the disappearance of meiocytes (arrows)

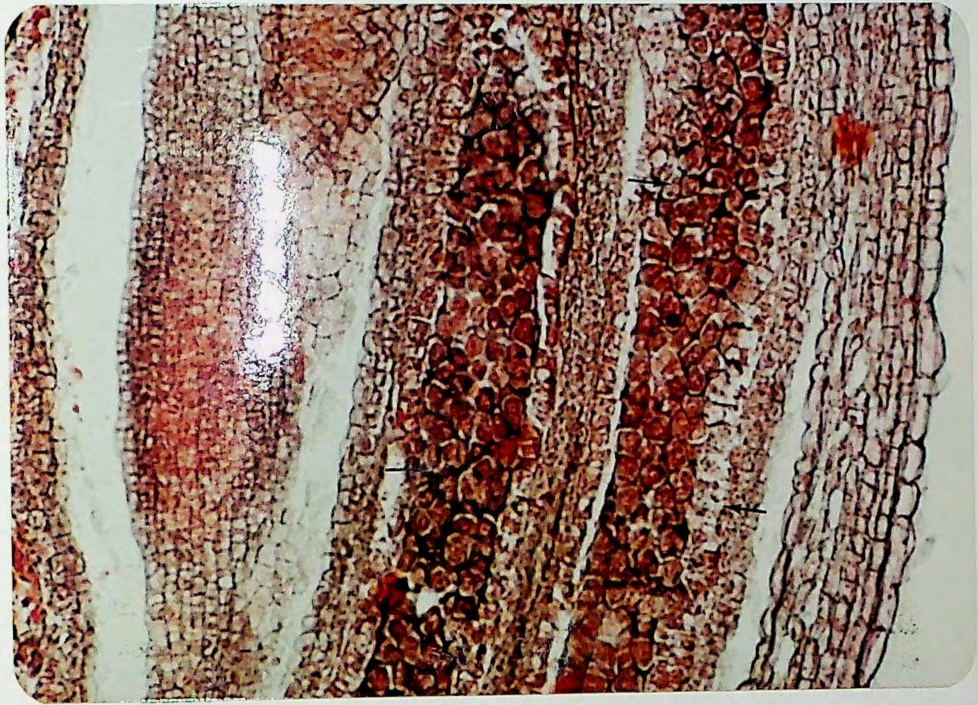


Fig 6.5

Fig 6.6 T.S. of MS anther showing aborted sporogenous mass (D) with undeveloped epidermis (E) and wall layers (W)

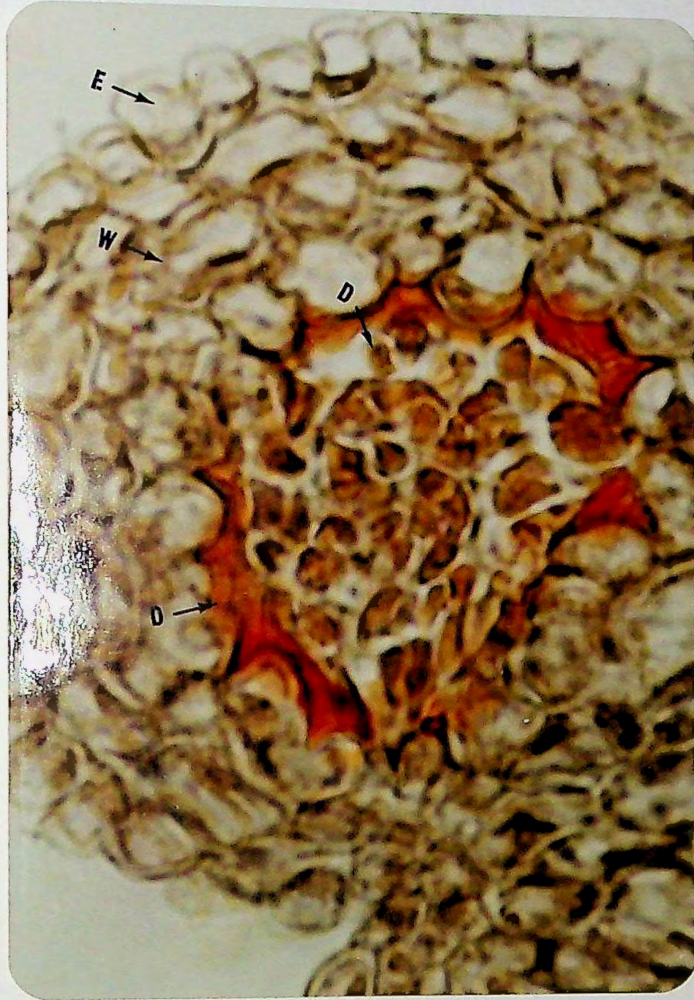


Fig 6.6

Fig 6.7 T.S. of MS anther with black crescent shaped degenerating sporogenous mass (arrows)

Fig 6.8 T.S. of MS anther with very deeply stained persistent tapetum (T) and thin walled microspores (arrows)

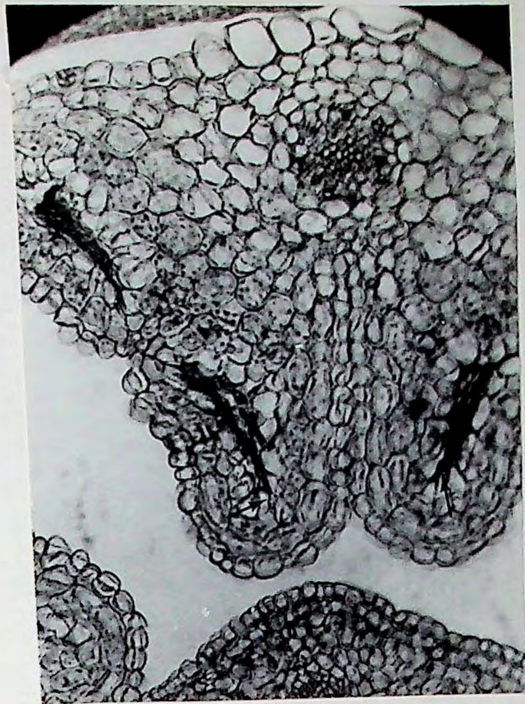


Fig 6.7

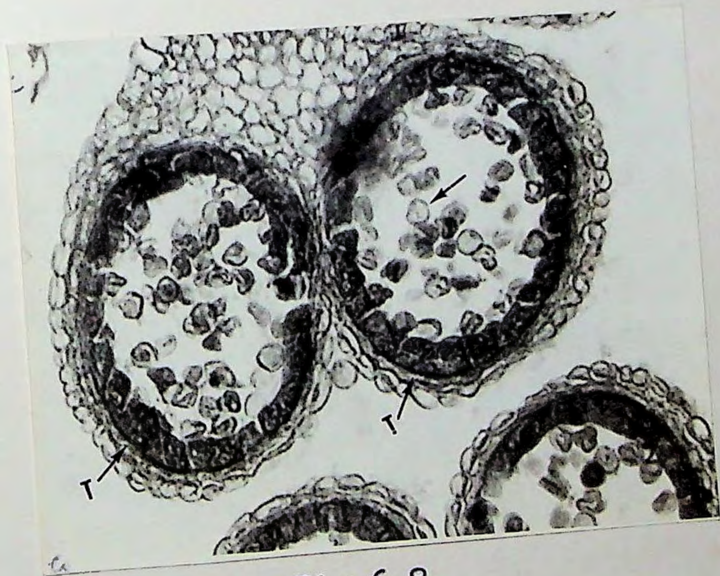


Fig 6.8

tapetum in post meiotic stages. The details of the differentiation of the wall layers and meiosis in the MMC's were the same as those of the normal MF plants (Section 5). The primary sporogenous cells in each locule of the anthers were much larger than those of the adjacent tissues. The primary sporogenous cells undergo further division to produce a large number of MMC's, meiosis proceeds normally and tetrad of microspores are formed. At the time of separation, the microspores were thin walled and uninucleate. In contrast to the MF anthers where all the microspores were separated and soon increase in size and became surrounded by an elaborate exine, the microspores in the MS anthers in tetrads or in separate conditions remained thin walled and begun to degenerate (**Fig. 6.8**). The tapetal cells in MF anthers start disappearing whereas in the MS anthers the tapetum persisted long after this stage as a deeply stained cellular body (**Fig. 6.9** and **6.10**). This persistence of tapetal cells in the MS anthers seemed to be the cause of disintegration and abortion of microspores.

As the anthers approach maturity, in MF flower, the two lobes on each side of the anther became confluent due to dissolution of wall layers between them. At the time of anther dehiscence all the anther wall layers including the tapetum disappear except the epidermis and the endothecium. Anther dehiscence takes place by a longitudinal slit on both sides of the anther.

Fig 6.9 T.S. of an MS anther loculus showing deeply stained persistent cellular tapetum (T) and degenerating microspores (P)

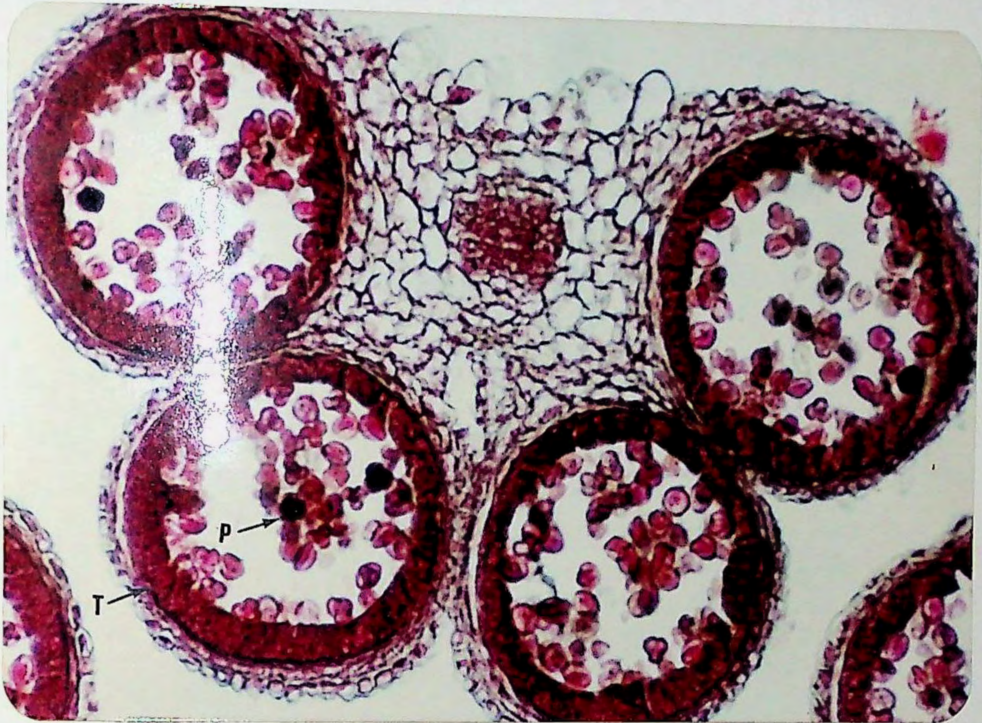


Fig 6.9

Fig 6.10 T.S. of an MS anther loculus (magnified) with degenerating microspore (P) and persistent tapetum (T)

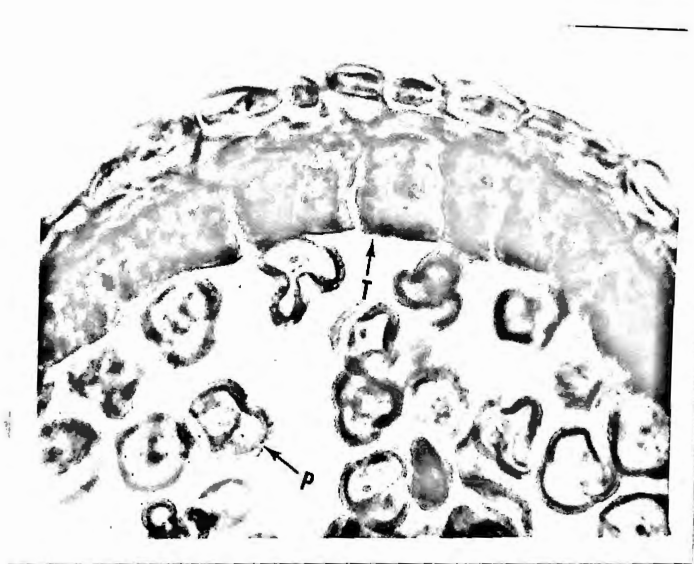


Fig 6.10

In contrast, in MS anthers the individual microspores and tetrads remained thin walled and gradually degenerate followed by the late breaking down of the cellular tapetum (Fig. 6.11). Aborted anthers in mature buds contain remnants of microspores and tetrads but no normal pollen grain (Fig. 6.12). The degenerated anthers often contain empty microspores with rudimentary exine.

6.3. Microsporogenesis in MS : Family BC–T–BH86/59/31–S/

As in other cases, in the MF plants of this family BC-T-BH86/59/31-S, typical microsporangia were formed early in the anther development. Each microsporangium consisted of four distinct tissues, epidermis, three to four parietal layers, a uniseriate tapetum and a central sporogenous mass. Each sporogenous mass appeared globose in cross-section and remained in this appearance throughout microsporogenesis.

The cytoplasm of the meiocytes and the tapetum of MS anthers were dense throughout meiosis (Fig. 6.13). There were many small vacuoles in the sporogenous cells. The tapetal cytoplasm was quite dense, there were several large vacuoles in each tapetal cell. During the end of meiosis, the locule enlarge. The microspores also enlarged and became more vacuolate, tapetum with dense cytoplasm persisted. The

Fig 6.11 T.S. of an anther loculus of MS plant with remnants of degenerated microspores (P) and disappearing tapetum (T)

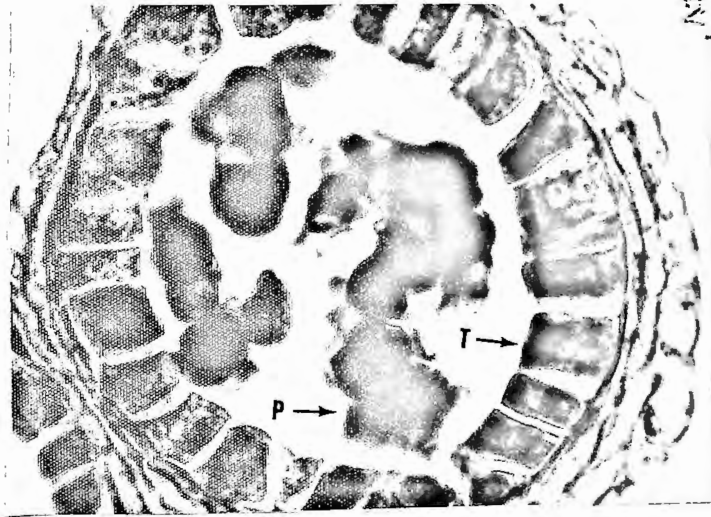


Fig 6.11

Fig 6.12 T.S. of anther loculus showing black sporogenous mass (B) and tapetum of MS plant, the wall layers not developed (W)

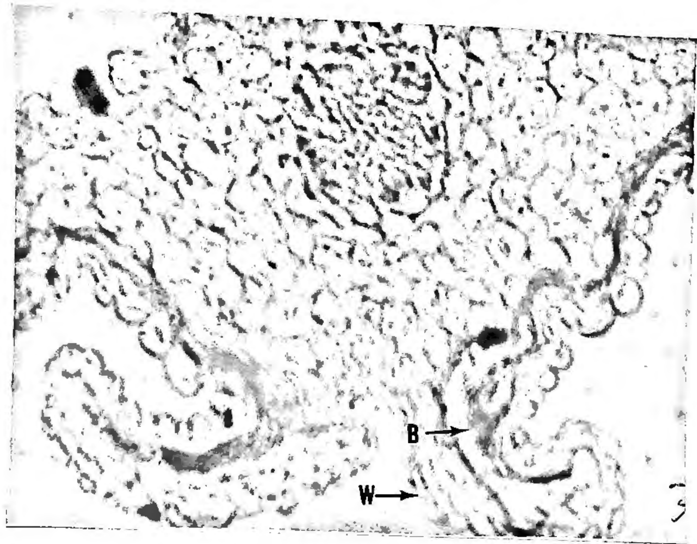


Fig 6.12

Fig 6.13 T.S. of anther of MS plant showing deeply stained degenerating meiocytes (B) and poorly developed epidermis (E)

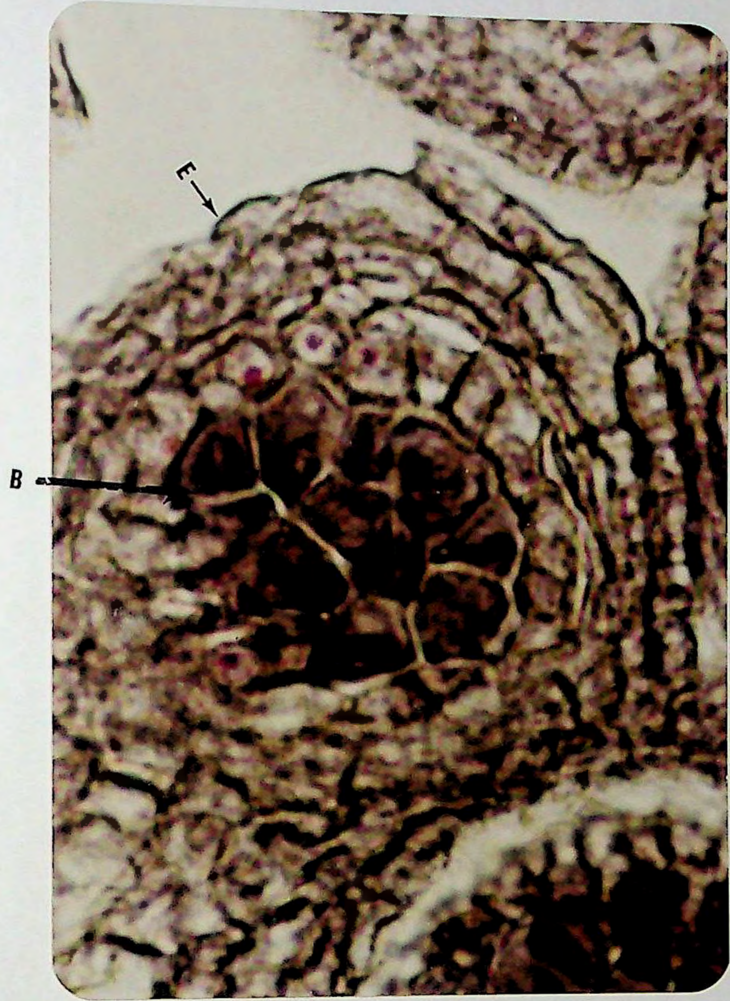


Fig 6.13

formation of normal pollen grains accompanied the degeneration of tapetum and the development of the endothecium.

In the MS plants, the early developmental stages are comparable to those of the MF lines. Anatomically too, both MS and MF plants are similar through the premeiotic stage. The anthers of the MS plants exhibited no evidence of disturbance in either the MMC's or the tapetal cells at the pre-meiotic stage. The tapetum appeared to be comparable to the tapetum of the MF anther at sporogenous stage (Fig. 6.13). However, before meiotic cell division, the cytoplasm of tapetal cells became much vacuolated and cell walls between adjacent cells broke down, resulting in a black fluid structure which invaded the meiocytes (Fig. 6.14). The meiocytes and their nuclei are normal in appearance at the beginning but the cytoplasm becomes very dark staining later (Fig. 6.14). Though the meiocytes had still retained their shape, the normal organization of the locule had broken down (Fig. 6.15). The wall layers fail to differentiate into well-organized epidermis, endothecium and middle layers, these remained ill-developed but intact while the tapetum and MMC's had become an unorganized, black mass (Fig. 6.15). At later stages, the black mass became compressed and was distributed at one end of the locule as a thin layer. Afterwards, the middle layers had also broken down, the anther remaining in this condition throughout flower development. Although intact, the endothecium failed

Fig 6.14 T.S. of anther of MS plant with thick black degenerated cytoplasm (B) in sporogenous cavity with large faint nuclei (N)

Fig 6.15 T.S. of MS anther with undeveloped wall-layers, tapetum and black unorganised sporogenous mass (arrows)

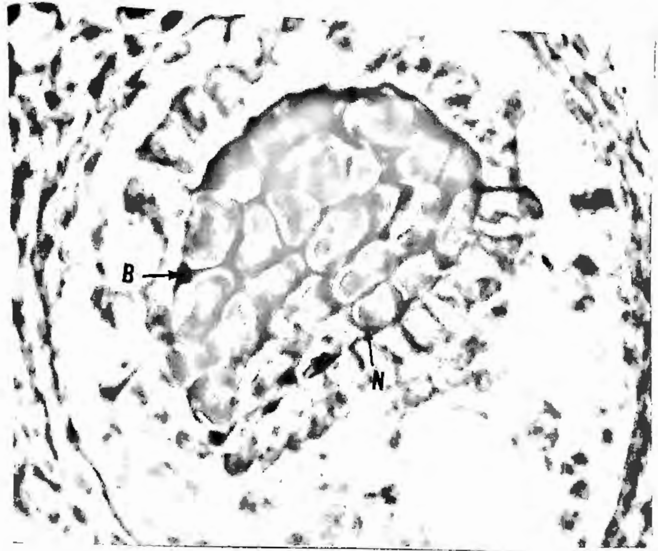


Fig 6.14

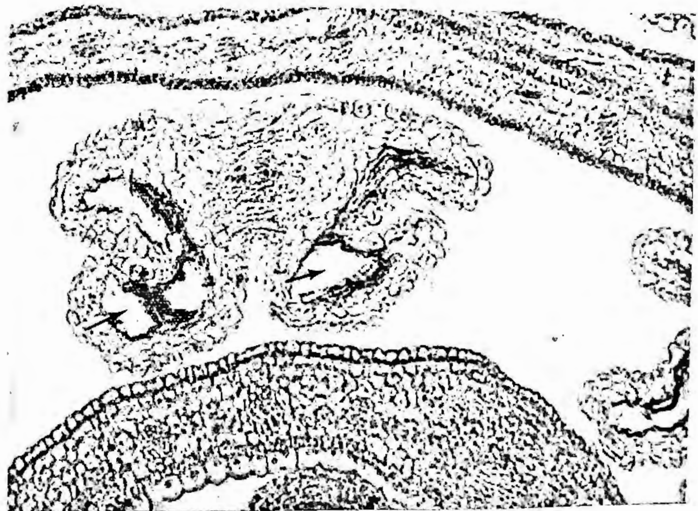


Fig 6.15

to develop normally and its walls did not develop thickenings and the loculi of anthers did not split open at the time of anthesis. The epidermal cells were unstretched and irregular in shape . At this time, the sterile anthers are smaller than fertile, whitish in colour and thread-like in appearance. In transverse section, the aborted anthers were crescent shaped and empty without any normal or aborted pollen grains (Fig.6.15).

6.4. Microsporogenesis in MS : Family BC-T-k86-19/3-S/

The microsporogenesis of the MS and MF anthers in this case is normal upto pre-meiotic stage. The tapetum differentiates normally but became abnormal in MS anthers before the onset of meiosis. The tapetal cells become hypertrophied (Fig. 6.16) and elongate radially. The radial elongation of the tapetal cells continues uninterrupted with the result that they occlude the anther cavity thus crushing the meiocytes completely (Fig. 6.17) in the MS anthers.

Endothecial cells in these MS anthers fail to develop and elongate radially and do not develop thick walls. The outer wall layers including the epidermis remained unorganized and loosely arranged (Fig. 6.17). When MS flowers mature, the whole of tapetum and the sporogenous tissue disintegrate completely (Fig. 6.16), the wall layers fail to develop normally, resulting in short, aborted anthers. The partition

Fig 6.16 T.S. of anther of MS plants with tapetal cells (T) behaving abnormally (elongating) with broken down sporogenous cells (S)

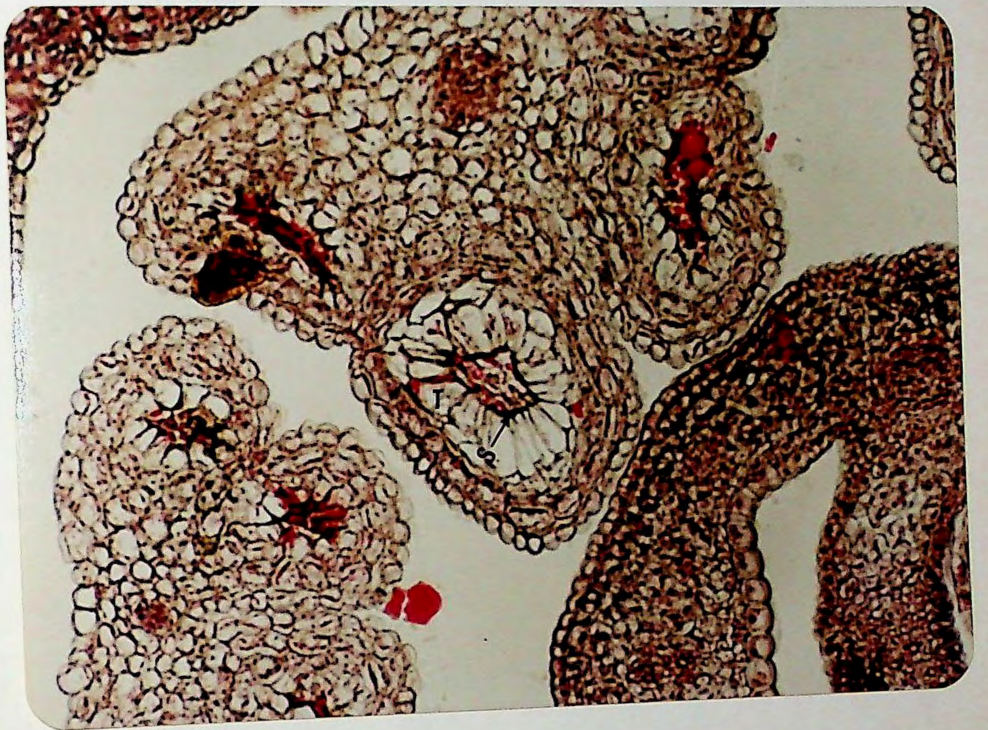


Fig 6.16

Fig 6.17 T.S. of M S anther with hypertrophied tapetum (T) and disintegrated sporogenous mass (S)

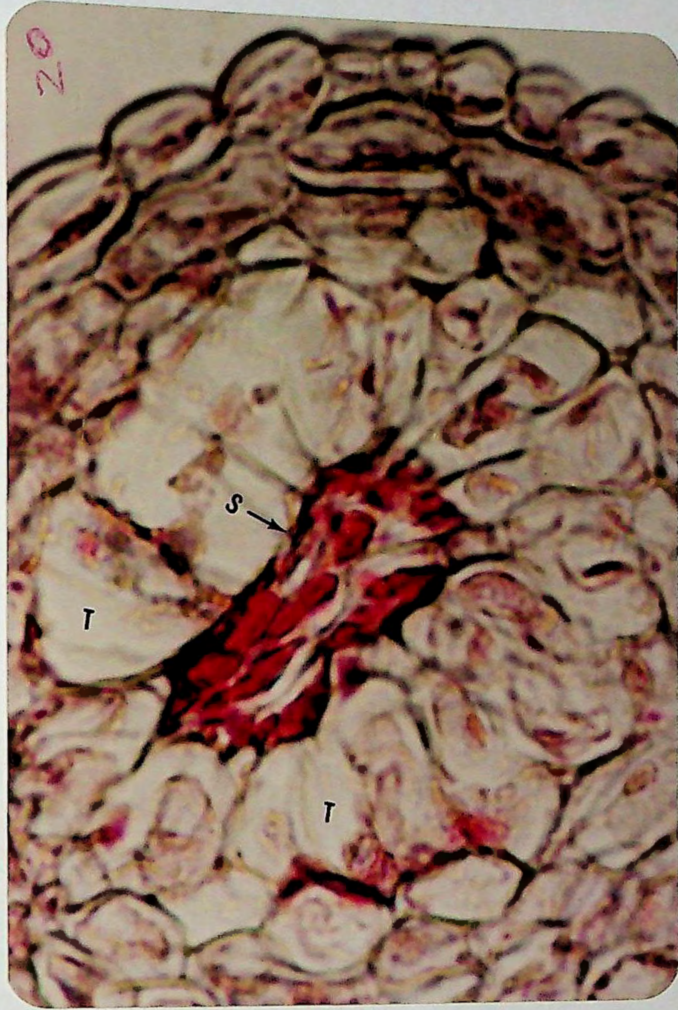


Fig 6.17

between neighboring loculi does not break. Owing to the empty cavity and reduced pressure of the wall layers, each loculus becomes cramped and sickle shaped. No aborted pollen grains or signs of sporogenous mass were found in later stages.

GENERAL DISCUSSION

7. General Discussion

7.1. Genetics of MS

The inheritance of male sterility in two cultivars of *Brassica campestris* was found to be monogenic and the sterility was due to recessive alleles. Whether the two sterility types (Sections 3.2 and 3.3) are controlled by the same gene or different genes cannot be determined due to lack of time and was not important for the present study. However, inter family crosses were made, further analysis and progeny testing can establish whether MS in the two cultivars studied are controlled by the same gene (*ms*) or by the two genes (ms_1 and ms_2).

Single recessive gene-controlled MS has been demonstrated in many flowering plants of diverse families like *B. campestris* Var. Yellow Sarson (Chowdhury and Das, 1967a); *B. campestris* Var. Brown Sarson (Chowdhury and Das 1967b); *B. napus* (Koch and Peters, 1953; Takagi, 1970); *B. oleracea* (Cole, 1957; Jensma, 1957; Johnson, 1958; Nieuwhof, 1961; 1968; North, 1961; Borchers, 1971). *Glycine max* (Brim and Young, 1971; Patil and Singh, 1976; Palmer, Winger and Albertsen, 1978); *Cucumis melo* (Bohn and Whitaker, 1949); maize (Beadle, 1932); tomato (Rick, 1948).

In Soybean (*Glycine max*), four independent recessive mutations of MS were identified in four different cultivars which were caused by the

same allele *ms*, (Palmer *et al.*, 1978).

However, in *Brassica oleracea* two different *ms* genes, ms_a and ms_b were obtained to cause MS (Nieuwhof, 1968). In *B. campestris* Var. Brown Sarson too, non-allelic genes were detected to control MS in self-incompatible and self-compatible cultivars (Chowdhury and Das, 1967a).

In maize (Zea mays) 12 different genes were found to cause MS (Beadle, 1932). Rick (1948) also reported non-allelic genes controlling MS in tomato.

Though many MS genes have been identified in flowering plants, but the mode of development and allelism tests in many taxa have not been carried out. Gottschalk and Kaul (1974) reported that microsporogenesis had been examined in 99 MS cases in 48 species belonging to 12 families. They noted very few examples of independent mutations that were allelic.

The occurrence of several different *Ms* genes offers possibilities of producing lines with a high percentage of male sterile plants by accumulations of these *ms* genes. Thus, plants with genotype ms_1 ms_1 pollinated with pollen from Ms_1 ms_1 will give progenies with 50% Male sterile plants, but male sterile plants of genotype ms_1 ms_1, ms_2 ms_2 pollinated by pollen from a male fertile double heterozygote, Ms_1 ms_1 Ms_2 ms_2 plant; will give progenies with 75% male sterile plants. This is advantageous for hybrid seed production. However, accumulation of *ms* genes is not easy to carry out.

Therefore further genetic analysis will probably establish different genes to be involved in the MS of the different types reported here.

7.2. Four different types of MS

The stages at which signs of events leading to abortion of microspores and male sterility separate four different types in the present material.

(a) abnormal behaviour initiated in the meiocytes before the initiation of meiosis followed by breaking down of tapetum.

(b) abnormal behaviour initiated due to the persistence of tapetum long after meiosis and microspore formation, resulting in the abortion of microspores or tetrads;

(c) abnormal behaviour in the early disintegration of young tapetal cells during meiosis followed by break down of meiocytes; and

(d) abnormal behaviour of tapetum before the onset of meiosis in hypertrophied growth of young tapetal cells crushing the meiocytes.

Each of the above types of activities leading to MS have been reported in various plants including one in ***Brassica campestris.*** But this is probably the first report of four different types occurring in the same genus.

The only cyto-morphological study conducted on MS in Yellow- and Brown-Sarson of *B. campestris* (Chowdhury and Das, 1967) indicated that persistent tapetum not degenerating long after the formation of microspore resulted in the MS in Yellow Sarson. On the other hand non-dehiscence of anthers, otherwise normal with viable pollen grains, due to abnormal anther wall resulted in MS in Brown Sarson.

In *Linum usitattissimum* too, the failure of the tapetal cells to degenerate at a proper stage, cause a failure in supply of substance required for the development of microspores into viable pollen grains (Dubey and Singh, 1965). In fact, the most frequent abnormality is the delayed degeneration of tapetum (Aalders and Hall, 1963; Webster and Singh, 1964).

In squash (*Cucurbita maxima*) where MS is also genetically controlled, absence of meiosis is the MMC's and subsequent degeneration of MMC's leads to sterility (Singh and Rhodes, 1961).

In GMS *Cucurbita maxima*, the tapetal cells become hypertrophied at the sporogenous cell stage. They crush the MMC's thus ceasing the development of the anthers in MS flowers (Chauhan, 1979). In *Cucumis melo*, similar hypertrophied behaviour of tapetum results in MS (Chauhan, 1979) at microspore tetrad stage.

In *Beta vulgaris* the tapetal cell walls break down and their protoplasmic contents flow into the anther loculus and mix with the

microspores thus causing MS (Chauhan, 1979).

Various types of events leading to MS suggest that different genes controlling different events in various steps of microsporogenesis and mutation in any of these genes can result in abnormal development and sterility at different stages. But it is obvious that abnormal development of the tapetum is frequently associated with MS in flowering plants.

7.3. Tapetum and MS

Substantial amount of literature can be found which stresses the impact of tapetum on pollen abortion thus resulting in male sterility (Edwardson, 1970; Kinoshita, 1971; Laser and Lersten, 1972; Mascarnenhas 1978). In the developing anthers of MS plants, meiosis was normal since tetrads are formed in both MS and MF lines in the same fashion. It is the abnormal behaviour of the tapetum which can be implicated as a causative agent in MS. It has long been suggested that the tapetum has a nutritive function during microsporogenesis (Vasil, 1967) and many studies note that abnormal tapetal behaviour almost always results in the failure of pollen to develop (Heslop-Harrison, 1972; Laser and Lersten, 1972; Overman and Warmke, 1972; Pritchard and Hutton, 1972).

Basically, the tapetum either degenerates too early or persists beyond its prescribed normal time of breakdown so that it is unable to

provide the developing sporogenous cells with substances required for pollen development. These substances have been variously interpreted as deoxyribosides, various metabolites, sporopollenin precursor and related wall compounds, and a number of enzymes, including callase. Evidences from the work of Horner and Rogers (1974) in pepper suggest that the tapetum is the layer that commands an important position to affect normal development. Abnormal development and functioning could easily upset the balance between these two tissues (tapetum and sporogenous cells), resulting in abortion (Heslop-Harrison, 1972).

In normal MF anthers, tapetum remains intact and functional up to the vacuolate pollen stage (Horner and Lersten, 1971; Heslop-Harrison, 1972;). In the MS pepper lines, tapetum looses its integrity just before meiosis and by the tetrad stage is highly vacuolate and enlarged. Thus, Horner and Rogers(1974) concluded that, MS in pepper is effected by conditions that control the development of the tapetum.

Without understanding the controlling mechanism involved in the initiation and synthesis of materials required, it can only be speculated that the malfunctioning of the tapetum, indicated by vacuolation, enlargement and lack of dense cytoplasm may already exerting its influence thereby arresting further development of microspores.

The breakdown of the spore mother cells before entering into

meiosis occurred in the present case also due to abnormal behaviour of the tapetum. The tapetal cells became vacuolated and invaded the locule. Vacuolation and enlargement of the tapetum has frequently been reported to occur in MS lines. In this type, the cellular nature of the tapetum remain s intact, and vaculation in each cell causes hypertrophy, some times to such an extent that the locule is occluded and developing microspores crushed. This has been observed in CMS beets (Artschwager 1947); corn (Chang, 1954), onion and radish (Nishi and Hiraoka, 1958), wheat (Chauhan and Singh, 1966), cucumber (Chauhan and Singh, 1968), sorghum (Overman and Warmke, 1972), and pepper (Horner, and Rogers,1974), sunflower (Horner, 1977).

In addition to the activity of the tapetum, another important factor which contributes to the MS in many diverse taxa is the mistiming of callose dissolution. Callose occurs as a special wall during meiosis (Heslop-Harrison, 1971, 1972., Horner and Rogers, 1974). Callose is considered to isolate the meiocytes and newly formed haploid spores from surrounding sporophytic tissues. In most taxa, the callose remains around the microspore tetrads until the exine is initially formed (Heslop-Harrision, 1971, 1972). At this time, when the anther has enlarged and the locular spaces are present, the callose is degraded, releasing the microspores from each other. In some studies early or late dissolution of callose is implicated in causing sterility (Izhar and Frankel, 1971;

Warmke and Overman, 1972).

Conclusion

In present study, as in others (Laser and Lersten, 1972; Horner and Rogers, 1974; Horner, 1977) suggested that in the sequence of events in the anther development, the failure in development of tapetum is a major cause in pollen abortion and male sterility. A number of studies now established it beyond doubt that the tapetum serves a major role during microsporogenesis and pollen development. However, the exact nature of this involvement, either through nutrition of the developing microspores or production of microspore wall components or both of these two, are still not clear. It is possible that these changes in the tapetum are initiated early in developmental stage, changes still undetected which later involve in the breakdown of the microspores.

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