CYTOPLASMIC MALE STERILITY IN RAPE (*BRASSICA NAPUS* L.)

A Thesis

Submitted to the Faculty

of

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by

ZHEGONG FAN

In Partial Fulfillment of the

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of

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ABSTRACT

Fan Zhegong, Ph.D., The University of Manitoba, October, 1985. Cytoplasmic male sterility in rape (Brassica napus L.). Major Professor, B. R. Stefansson.

Four male sterility systems in rape (Brassica napus L.) were investigated. The studies involved cytology, histology, inheritance of sterility and fertility restoration, and the effect of temperature on the expression of male sterility.

The mur male sterility system was developed by transferring the genome of B. napus into the cytoplasm of Diplotaxis muralis by means of backcrossing. Cytological examination of plants from the last backcrossed generation indicated that an extra chromosome was present in the male sterile plants and this extra chromosome appeared to be the cause of the male sterility.

The ogu cytoplasmic male sterility (cms) system is conditioned by the sterility inducing cytoplasm from radish (Raphanus sativus L.). Histological examination of stamen ontogeny of male sterile plants with the ogu cytoplasm revealed that the development of microspores was arrested at the uninucleate stage and the abortion of microspores was associated with the abnormal vacuolation of the tapetum. The ogu male sterility is stable and is not influenced by temperature. Studies of fertility restoration indicated that 32 strains tested were all maintainers for the ogu cytoplasm.

The nap cms system possesses the sterility inducing cytoplasm of B. napus. Histologically, this male sterility is associated with the lack of differentiation of archespores. The nap male sterility is not stable under high temperatures. The male sterile plants converted to partially male sterile and male fertile after treatments at 26/20 and 30/24° C (day/night) for seven days, respectively. Fertility of male sterile plants with the nap cytoplasm was restored by most strains tested. However, Bronowski was a partial maintainer for the nap male sterility. Lergo was heterogeneous for genes conditioning maintenance and restoration of this male sterility. The inheritance study suggested that both Karat and Westar possessed a single dominant gene for the restoration of fertility of the nap male sterile plants.

The pol cms system is conditioned by the cytoplasm of cultivar Polima which was obtained from Poland. Examination of stamen development indicated that the lack of archesporial differentiation was responsible for the male sterility. The expression of the pol male sterility can be influenced by temperature. The male sterile plants became partially male sterile after treatment at 30/24° C (day/night) for seven days. The fertility of male sterile plants with the pol cytoplasm was not effectively restored by any strains tested. However, a fertility restorer gene found in Brassica juncea (Zem) has been transferred into B. napus.

Two types of monosomic plants were identified in the backcrossed progenies involving interspecific crosses and were designated as mono-1 and mono-2, respectively. At diakinesis, chromosome configurations of 18 II + 1 I and 17 II + 1 III were observed in pollen mother cells of

both types of monosomics. However, frequency of trivalent formation was higher in mono-2 than in mono-1. The mono-1 plants produced large and small sized pollen grains, while pollen grains produced on mono-2 plants were uniform in size.

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INTRODUCTION

Rapeseed, which is the seed from the species Brassica napus L. and Brassica campestris L., is a major oil crop in China, India, Canada, the United Kingdom, and several European countries. In the last 20 years, Canada has been a major producer and exporter of rapeseed in the world. In the period from 1982 to 1984, the acreage of rapeseed increased from 1.776 to 2.990 million hectares with a total production from 2.225 to 3.246 million metric tonnes (Cereals and Oilseeds Review, Statistics Canada 1985). Rapeseed is exported as seed, oil, and meal. Japan has been the major buyer of Canadian rapeseed, usually taking about 80% of Canada's total exports. The remainder of the exports go to several European and Asian countries. Oil is exported to Hong Kong, India, and the United States. West Germany, Netherlands, and South Korea were the major importers of the meal during the last three years.

It has been estimated that the world export market for Canadian rapeseed could be 4 million metric tonnes by 1990, since the consumption of vegetable oil has been increasing at a rapid rate (Horner 1980). Although the world production of vegetable oil has expanded rapidly, Canadian rapeseed is very competitive in the export market, due to its improved quality. Canadian plant breeders have reduced the erucic acid content in the oil and glucosinolate content in the meal. Cultivars with less than 5 % erucic acid and 30 micromoles of glucosinolate per gram of dry meal have replaced all traditional varieties and the product

has been named "canola" to distinguish its high quality (Stefansson 1983).

In addition to improving quality, plant breeders have been improving the yield of cultivars using conventional breeding methods. However, progress toward increased seed yield has been slow. Significant heterosis for seed production in the first generation of intervarietal hybrids has been reported in the past (Olsson 1954, Schuster 1969, Thompson 1973). Recent investigations indicated that F_1 hybrids could yield from 40 to 60% more than their parents or current cultivars (Schuster and Michael 1976, Shiga 1976, Morice 1978, Buson 1980, Guan 1980, Hutcheson et at. 1981, Sernyk and Stefansson 1983). Thus, hybrid cultivars would provide a substantial increase in seed yield of rapeseed.

Efficient production of hybrid cultivars requires a mechanism of pollination control which could operate effectively under field conditions. Several pollination control mechanisms, including cytoplasmic male sterility (Shiga 1980), self-incompatibility (Thompson 1979, Fu 1981), and application of male gametocides (Van Der Meer and Van Dam 1979), have been considered for hybrid production in rapeseed. It appears that cytoplasmic male sterility is more feasible for developing hybrid cultivars than the other two mechanisms.

Several cytoplasmic male sterility (cms) systems have been reported in Brassica napus ; they are the ogu, nap, and mur cms systems (Shiga 1980). The ogu cms was developed by transferring the genome of B. napus into a male sterility inducing cytoplasm of radish (Raphanus sativus L.)

discovered by Ogura (1968) (Bannerot et al. 1977). The nap cms was discovered in F_2 generations from intervarietal crosses of rape and involved the male sterility inducing cytoplasm occurring in B. napus (Shiga and Baba 1971, 1973, Thompson 1972). The mur cms was obtained by the transfer of the nucleus from B. napus to the cytoplasm of Diplotaxis muralis L. (Sernyk 1982). Another cytoplasmic male sterility system was developed from a rape variety Polima (Fu 1981). This cytoplasm was designated as the pol cytoplasm in this investigation.

The objective of this study was to evaluate the four cms systems, specific studies designed to examine floral morphology and histology of stamen development; to determine patterns of fertility restoration and sterility maintenance; to study the inheritance of fertility restoration, if restorers could be obtained for each system; and to determine the effect of temperature on the expression of the sterility. The information obtained was used to evaluate the potential of the four cms systems for the development of hybrid cultivars. During the investigation, a few monosomic plants were discovered in backcrossed progenies involving interspecific crosses. The cytology of these monosomic plants was also investigated.

LITERATURE REVIEW

Taxonomy of Brassica napus and its relatives

The genus Brassica contains a group of species cultivated widely as sources of seed oil, vegetables, condiment, and fodder. Cytotaxonomic relations among these species have been determined (U 1935) and are outlined by the triangle of U (Fig. 2.1). Three species, B. campestris, B. nigra, and B. oleracea, are elementary diploids and have been assigned as A, B, and C genomes, respectively. The other three species, B. napus, B. juncea, and B. carinata, are amphidiploids which originated from interspecific crosses between the three elementary diploids.

The seeds from B. napus, B. campestris and sometimes other species of Brassica are called rapeseed. B. napus is usually referred to as rape. Cultivars of this species have been developed for fodder and oil seed production. Annual and biennial forms occur within the species. The winter form of oil seed rape is grown extensively in Europe and Asia. All the cultivars grown in Western Canada are annual forms (Stefansson 1983). Seed yields from winter rape are usually higher than those from summer rape.

Radish (Raphanus sativus L.) is a vegetable crop grown for the enlarged succulent root. The plants are usually self-incompatible and are pollinated by insects. R. sativus has 18 chromosomes and is designated as R genome. Studies of meiotic chromosome pairing behavior

Figure 2.1: The triangle of U illustrating the relations between six species of Brassica.

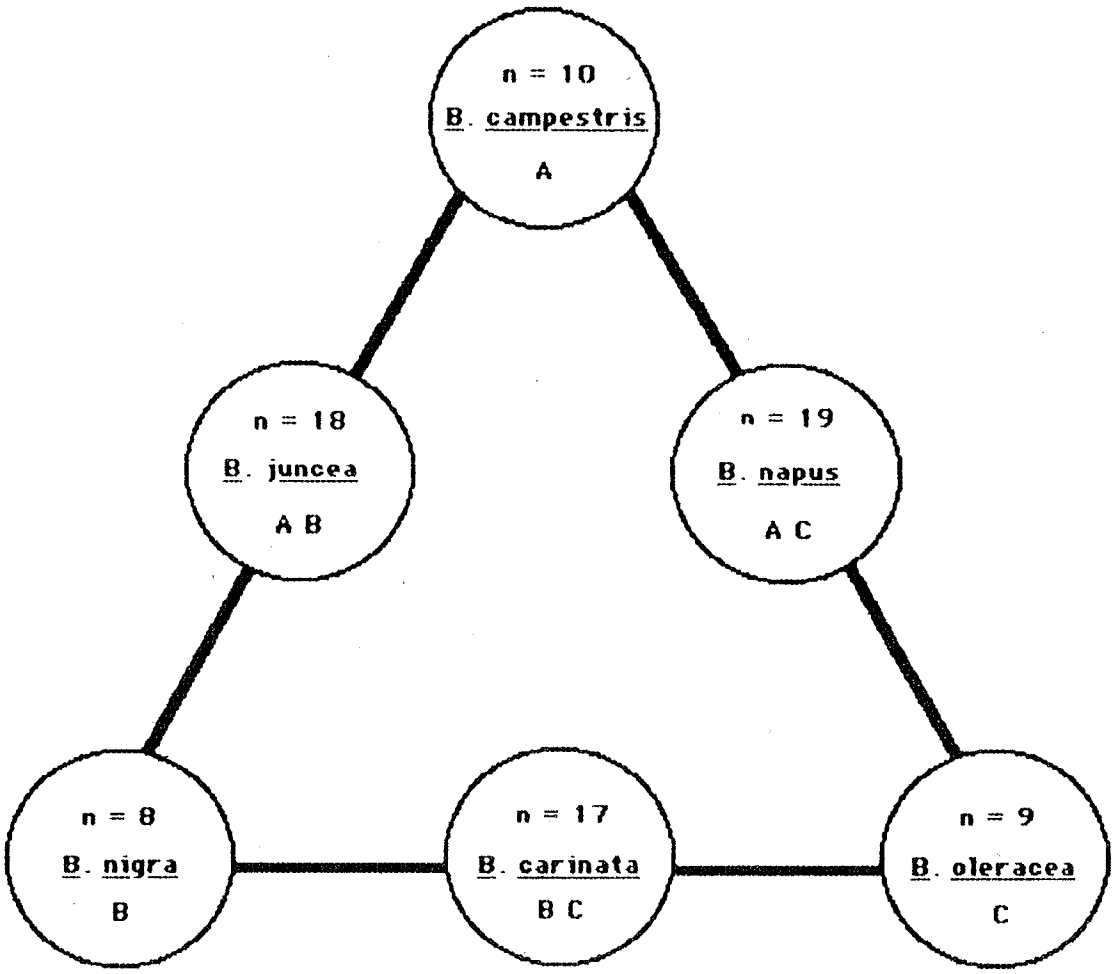


Fig. 2.1. Triangle of U, illustrating the relations between six species of Brassica

in Brassica-Raphanus hybrids have shown that the R genome is partially homologous with the A and C genomes of Brassica (Prakash and Hinata 1980). Cytoplasmic male sterility was found in radish (Ogura 1968) and the sterility inducing cytoplasm of radish was utilized to produce male sterile lines in Brassica crops (Shiga 1980).

Sand rocket (Diplotaxis muralis L.) is a short-lived weed, which occurs in annual, biennial or perennial forms and is mainly distributed in Southern and Central Europe (Tutin et al. 1964). D. muralis (2n=42) is allotetraploid species derived from D. tenuifolia (2n=22) and D. viminea (2n=20) (Harberd and McArthur 1972). The cytoplasm of D. muralis has been used to produce male sterile lines in B. campestris (Hinata and Konno 1979).

Heterosis

Shull (1914) introduced the term heterosis to describe a common phenomenon, the increase in vigor and productivity of F_1 hybrids. Allard (1960) and Simmonds (1981) defined heterosis as a converse of inbreeding depression. Heterosis can also be defined as the improvement of F_1 hybrids over the mid-parent, the best parent or a set of standard cultivars. All these definitions contain the same concept that F_1 heterozygotes are superior to the corresponding homozygotes in respect to some measurable attributes. In current usage, Sprague (1983) indicated that heterosis and hybrid vigor were essentially synonymous and were used interchangeably.

Several theories have been advanced to explain how heterozygosity leads to heterosis. These are dominance of favorable factors, overdominance, and intergenomic (mitochondrial, chloroplast, and nuclear) complementation.

The hypothesis of dominant favorable factors, proposed by Keeble and Pellew (1910), Bruce (1910), and Jones (1917), assumed that favorable alleles were dominant, while deleterious alleles were recessive. The F_1 hybrids possessed all dominant alleles contributed by both parents. Recessive alleles from one parent were hidden by dominant alleles from the another and vise versa in the F_1 heterozygotes. According to this theory, hybrid vigor was caused by the complementary effects of dominant alleles at many loci. If this is true, inbred lines containing all homozygous dominant alleles could be obtained through breeding and selection, and consequently vigor could be permanently fixed in these lines. However, this has not been achieved in plant breeding. Jones (1917) explained this discrepancy by invocation of genetic linkage. He indicated that a linkage group included many dominant and recessive alleles and accumulation of all dominants in a gamete through precise recombinations with recessives would be extremely rare.

An alternative hypothesis for heterosis is overdominance originally proposed by Shull (1908) and East (1908). It presumed that heterosis was due to stimulating effects arising from heterozygosity. East (1936) further explained this concept using a divergent alleles model. It states that a series of divergent alleles involves different physiological functions and heterozygotes with these divergent alleles at many loci perform these functions more effectively than both

corresponding homozygotes. Although the overdominance theory has been difficult to demonstrate, evidences from studies of single-gene heterosis (Whaley 1952) and factorial analysis of isogenic lines (Fasoulas and Allard 1862) indicated that heterosis was partly conditioned by overdominance. A recent review of the dominance and overdominance theories has been presented by Sprague (1983).

The third theory, intergenomic complementation, emphasizes that heterosis is regulated by interactions among nuclear, mitochondrial, and chloroplast genomes, rather than solely conditioned by nuclear genes (Srivastava 1983). The involvement of cytoplasmic factors in heterosis can be demonstrated by comparisons of reciprocal crosses involving inbred lines (Khera and Bhalla 1976), by lines with the same cytoplasm but different genetic backgrounds (Rozoriteleva and Beletskii 1979), and by lines with the same genetic background but different cytoplasms (Rao and Fleming 1978). It is known that a few key enzymes in the cellular energy system, such as cytochrome oxidase and adenosine triphosphatase in mitochondria and ribulose-1,5-biphosphate carboxylase/oxygenase in chloroplasts, are jointly coded by cytoplasmic and nuclear genes (Gillham 1978). Srivastava (1983) proposed that some key parts of cellular metabolism were co-ordinately regulated by multigenomes and hybrid vigor was due to elevation of a rate-limiting step in many biochemical systems.

Significant heterosis in oil seed rape has been known for some time. Olsson (1954) studied the F_1 hybrids of a cross between a Swedish and a Japanese cultivar, and found that plant height exhibited a statistically significant heterosis; seed yield of the F_1 plants was also higher than those of both parents, but the difference was not significant.

Schuster (1969) observed that yield of a winter rape cultivar decreased gradually as inbreeding was successively exercised. The mean yield of 73 crosses made by hand pollination with different inbred lines derived from that cultivar was 30% higher than that of their progenitor. The individual F_1 hybrids showed marked differences in yield.

Takagi (1970) obtained a monogenic recessive male sterile mutant in rape by gamma-irradiation of growing plants. The F_1 hybrids made by pollination of the genic male sterile plants with pollen from a number of Japanese cultivars gave seed yields 30 to 70% more than the average of the parents. Heterosis for oil content of hybrids was also observed.

Schuster and Michael (1976) investigated the level of inbreeding depression and hybrid growth, and the possibility of developing synthetic varieties in winter rape. They found that approximately 20% of F_1 hybrids averaged 17% higher in seed yield than standard winter rape cultivars.

Shiga (1976) evaluated the appearance of 62 hybrids involving European and Japanese winter rape cultivars. Heterosis was observed for seed yield, 1000 seed weight, length of inflorescence, and number of primary branches. He also produced 131 hybrids using cytoplasmic male sterile plants and various pollinators. The F_1 hybrids displayed yield gain up to 49% over the male parents in the crosses.

Buson (1980) conducted an incomplete diallel experiment involving 25 winter rape lines, from which the 130 single-cross hybrids were obtained. The hybrids showed an average of 23% yield gain above the inbred lines. Hybrid vigor was also observed for leaf area, plant

height, number of silique per plant, and number of seeds per silique. Reciprocal differences were significant for yield, leaf area, and date of flowering. Combining ability estimates revealed that general combining ability was more important than specific combining ability in controlling heterosis in these inbred lines.

Sernyk and Stefansson (1983) made a number of F_1 hybrids by crossing foreign cultivars with the Canadian cultivar Regent. The degree of heterosis for seed yield in these F_1 hybrids was examined in replicated yield trials for two years. The seed yields from the F_1 hybrids of crosses between Marnoo and Regent, and Karat and Regent exceeded those of Regent by 38 and 43%, respectively. Heterosis was also observed for total dry matter and harvest index.

Olivieri and Parrini (1983) used 20 winter and spring rape cultivars to produce 420 F_1 progenies in a diallel experiment. Analysis of variance for seed yield and its components revealed that general combining ability accounted for most of the variations, while specific combining ability and maternal effects were small. The combinations between winter and spring rape cultivars showed the highest heterosis for seed yield.

Cytoplasmic male sterility in rape

Male sterility can be defined as the incapacity of a plant to produce or to release functional pollen (Sneep et al. 1979). The male sterile phenotype may be expressed as the absence or faulty differentiation of male sex organs, microspore abortion, and nondehiscence of anthers.

According to the inheritance pattern, male sterility can be classified into two types, i.e. genic and cytoplasmic (Frankel and Galun 1977). Genetic male sterility is conditioned by recessive nuclear gene(s) (msg) and a fertile restoring cytoplasm is not available for this type of male sterility. The cytoplasmic (or cytoplasmic-genetic) male sterility is conditioned by a male sterility inducing cytoplasm and recessive gene(s) rf. These genes do not induce male sterility in the fertile cytoplasm. In this case, two or more different cytoplasms and two or more different alleles exist in a species.

Cytoplasmic-genic male sterility occurs widely in the plant kingdom and its inheritance has been documented for about 150 species (Edwardson 1970). The control mechanism of this type of male sterility in the simplest situation can be explained on the basis of two kinds of cytoplasm and a pair of alleles. The two alternative cytoplasms are usually described as sterility inducing (S) and normal fertile (N or F) plasmatypes, and the two alleles are usually designated rf, if recessive and unable to restore fertility, and Rf, if dominant and able to restore fertility. The male sterility is produced by the homozygous recessive genotype (rf rf) with the (S) cytoplasm. Whenever the cytoplasm is the (N) plasmatype or the Rf alleles exists, fertility is assumed.

In practice, the term cytoplasmic male sterility, and its abbreviation cms, is commonly used to refer to cytoplasmic-genic male sterility. This convention is also followed here. Cytoplasmic male sterility may occur spontaneously in a population. It can also arise from interspecific hybridization (Edwardson 1970). In the latter case, a restorer gene(s) for pollen fertility is usually found in the species which contributes the cytoplasm (Smith 1968).

Several male sterility inducing cytoplasms are available in B. napus. Shiga and Baba (1971, 1973) reported that male sterile plants occurred in F_2 generation of a cross between Chisaya-natane (female) and Hokuriku 23. The reciprocal cross demonstrated that this male sterility was based on a sterility inducing cytoplasm. The male sterile plants were shorter, and had lower seed fertility than their fertile counterpart. Abnormal floral characteristics of those plants were small buds, rugose petals, short filaments and poorly developed anthers. Partially sterile plants were also observed with varying degrees of morphological abnormalities.

Thompson (1972) crossed a number of rape strains with the spring cultivar Bronowski. Male sterile plants were only found in F_2 generations of those crosses using Bronowski as a male parent. The plants had small petals and a tendency toward late maturity. He proposed that Bronowski was homozygous for a recessive allele (rf) for pollen fertility but possessed a fertile cytoplasm. The other strains tested had a sterile cytoplasm and the dominant allele (Rf) for restoration of fertility.

Shiga (1976) and Shiga et al. (1983) compared the fertility restoration pattern of these two cms systems discovered by Shiga and Baba (1971) and Thompson (1972), respectively. They found that fertility restorers and sterility maintainers for the two systems were basically the same, indicating that they possessed the same type of male sterile cytoplasm of B. napus. Shiga (1980) designated this cms system as the nap cms.

The inheritance of fertility restoration for the nap cms has been investigated by several authors. Thompson (1972) reported that the restoration was governed by a single dominant gene. Shiga (1976) and Shiga et al. (1983) found that dominant restorer genes in European and Japanese varieties ranged from 1 to 4, depending on the variety tested. Rousselle and Renard (1978, 1979) crossed 20 spring rape strains with Bronowski. Data of F_2 segregation indicated that the male sterility in Bronowski was controlled by homozygous recessive alleles at two loci. Sernyk (1982) studied the fertility restoration for the nap cms using the cultivar Regent. He found that dominant alleles at five loci were involved in the restoration. The inheritance of fertility restoration for the nap cms appears to be rather complicated and many restorer genes appear to be involved.

The nap cms can be maintained by self-pollination since the male sterile plants may produce functional pollen under high temperatures (Shiga 1976, Thompson 1972). For breeding purposes, it is desirable to develop a maintainer line using cultivars with the fertile cytoplasm, such as Bronowski (Thompson 1972), Isuzu-natane, Narasaki-natane (Shiga 1976), Brio and Ceska (Rousselle and Renard 1978).

Ogura (1968) found male sterile plants in an unidentified variety of Japanese radish (Raphanus sativus) and established that this male sterility was conditioned by a male sterile cytoplasm. On the sterile plants, the flower buds which sometimes did not open were small and the style was often crooked. Bonnet (1975, 1977) introduced this male sterility into European radish cultivars and used it to produce commercial F_1 hybrids. The genomes of B. oleracea and of B. napus were

transferred into the sterile radish cytoplasm by repeated backcrosses and cms lines in the two species were established (Bannerot 1975, 1977). Shiga (1980) classified this male sterility inducing cytoplasm in B. napus as the ogu cytoplasm.

The rape plants with the ogu cytoplasm exhibited severe chlorophyll deficiency when grown at temperature below 12° C. Furthermore, the number of nectaries was reduced and were non-functional (Heyn 1979, Rousselle 1979, 1983). Pelletier et al. (1983) produced somatic hybrids between the ogu male sterile plants and rape cultivar Brutor through protoplast fusion. Some of regenerated plants were male sterile and green (no chlorophyll deficiency), and had normal nectaries.

All rape cultivars tested were unable to restore the fertility of the ogu cms (Heyn 1979, Rousselle 1979, 1983). It is generally accepted that restorer gene for the ogu cms is not present in B. napus. However, some European radish cultivars can restore the fertility of the ogu cms (Bonnet 1975). It may be possible to introgress restorer genes from Raphanus sativus into B. napus.

Hinata and Konno (1979) reported that a cms line in B. campestris was established by transfer of the B. campestris genome into the cytoplasm of Diplotaxis muralis by means of repeated backcrosses. The male sterile plants were characterized by two nectaries, narrow petals, and nondehiscent anthers which contained a small amount of pollen. Shiga (1980) observed that F_1 hybrids between D. muralis and B. napus (Norin 16) were male sterile, and designated this male sterility as the mur cms. He also indicated that no restorer gene for the mur cms was available in B. napus strains used in his experiment.

Fu (1981) reported that male sterile plants occurred spontaneously in the rape variety Polima originated from Poland. This male sterility has been designated as the pol cms. The pol cms has been studied rather extensively in China. Most rape strains were maintainers and only one variety, Italy, was able to restore the fertility of the pol cms.

Histology of stamen development in male sterile plants

Comparative studies of stamen ontogeny in male fertile and male sterile plants have frequently been conducted to elucidate cellular causes of male sterility. Literature on anatomy and cytology of androecium development in cytoplasmic and genic male sterile plants have been reviewed by Laser and Lersten (1972), Gottschalk and Kaul (1974), and Frankel and Galun (1977). They indicated that male sterility could be due to male sex suppression, sex reversion, breakdown of microsporogenesis, abortion of microspores, and nondehiscence of anthers.

Male sex suppression involves the failure of stamen differentiation due to abnormalities in the differentiation of the growing points during flower formation. Stamenless and antherless male sterile mutants have been reported in tomato (Bishop 1954, Hafen and Stevenson 1958) and cotton (Allison and Fisher 1964).

Sex reversion involves a phenomenon where male sex organs are transformed into female organs. There are several types of the sex reversion: sterile anthers may develop stigma-like extension at the distal end (stigmoid); stamens may be transformed into pistils

(pistilloid); sterile anthers may become carpel-like with typical stigma and style or bearing external ovules (carpeloid). Aberrant differentiation of the epidermal and primary parietal layers may lead to petaloid anthers. Many studies indicate that the sex reversion is related to the cytoplasmic and genic interaction (Meyer 1966, Pearson 1972).

Microsporogenesis may breakdown due to a number of aberrations which occur during meiotic division. Absence of pairing of homologous chromosomes, precocious separation of homologous chromosomes, and lack of terminalization of chiasmata during the first meiotic prophase were reported in a number of species (Gottschalk and Kaul 1974). Failure of cytokinesis after telophase I and irregular spindle orientation after metaphase II may lead to male sterility. Failure of release of microspores from tetrads due to lack of enzymatic digestion of callose may cause degeneration of microspores (Frankel and Galun 1977).

The abortion of microspores is very common in cytoplasmic-genic male sterile plants and frequently associated with abnormal behavior of the tapetum (Laser and Lersten 1972). The tapetum is a layer of cells located between sporogenous cells and anther wall tissue. Because of its critical cellular position, the tapetum is considered to play an important role in regulation of supply of nutrients and other essential metabolites to developing microgametocytes (Echlin 1971). The development of microspores is usually arrested at mononucleate stage, and then degeneration of microspores takes place. This degeneration is often associated with early collapse of the tapetum and abnormal tapetal vacuolation (Laser and Lersten 1972). Lack of accumulation of food reserves due to non-degenerate tapetum may also cause cessation of

further development of binucleate microspores in cytoplasmic male sterile barley (Schooler 1967).

Failure of anther dehiscence resulting in male sterility is not common in cytoplasmic male sterile plants. In some genic male sterility, nondehiscence of the anthers is associated with abnormal differentiation or functioning of the stomium. The stomium may be absent. In some cases, stomal cells may be tenacious and pressed by hooked petals which do not allow opening of corolla (Frankel and Galun 1977).

In B. napus, histological investigations of anther development in the ogu and the nap cms have beed reported. Ogura (1968) reported that pollen degeneration in the ogu cms in radish occurred at the microspore stage, and the degeneration appeared to have some relations with the early collapse of tapetal tissue. Bartkowiak-Broda et al. (1979) observed a deeply stained zone in sterile anthers of the ogu cms in rape. Since cell structure in the zone was not distinguishable, it was proposed that the zone consisted of degenerated materials of tapetum and microspores. They suggested that microsporogenesis stopped at tetrad stage. Shiga and Baba (1973) reported that differentiation of sporogenous cells and tapetum did not take place in sterile anthers of the nap cms plants, and suggested that inhibition of anther development occurred between stages of carpel differentiation III and male archesporial differentiation I. Little information is available regarding ontogeny of stamen development in the pol and the mur cms plants.

Influence of temperature on expression of cytoplasmic male sterility

Expression of cytoplasmic male sterility is conditioned by an intricate balance of interactions among cytoplasmic and nuclear hereditary systems and environment. Therefore, it is vulnerable to changes of environmental conditions. Several environmental factors, including temperature, photoperiod, water stress, and nutrient supply, can influence male sterile phenotypes (Edwardson 1970). Among these, temperature appears to be the major factor that strongly interacts with fertility restoring mechanism of cms genotypes.

Meyer (1969) reported that expression of male sterility in cotton (Gossypium hirsutum) with the cytoplasms from G. anomalun and G. arboreum varied in response to external environment. Daily maximum temperature 15 to 16 days before anthesis affected the sterility more than any other aspect of the external environment. High temperature increased the level of the sterility. Marshall et al. (1974) studied the same cms strains of cotton in a controlled environment. The male sterility was affected by both temperature and day length, but the effect of temperature was much stronger than that of day length. The sterility increased with increasing temperature and day length, and day temperatures above 33° C were essential for the consistent expression of the male sterility.

Marrewijk (1968) found that expression of male sterility in clones of cms Petunia was dependent upon environmental conditions, especially temperature. The sterile clones became fertile at 18 and 21° C, and this fertility decreased markedly when temperatures were below 12° C.

Izhar (1975) observed a critical stage of anther development of cms Petunia, at which sterile plants were highly sensitive to temperature. Cytological examination indicated that this critical stage was between premeiosis II and early prophase.

In B. napus, the nap and the pol cms have been reported to be susceptible to high temperature. Thompson (1972) and Shiga (1973) observed that the nap cms plants produced functional pollen at late stages of flowering under the influence of high temperatures. Fu (1981) reported that the pol cms plants became fertile when temperature was high. Temperature sensitivity of these cms systems may present problems for hybrid seed production. However, selection of lines with improved stability under warm conditions is possible.

MALE STERILITY IN BRASSICA NAPUS L. ASSOCIATED WITH AN EXTRA CHROMOSOME

Abstract

Male sterility was investigated in backcross populations from hybrids between Diplotaxis muralis and Brassica napus using the former as the female parent. The F_1 was male sterile and low frequencies (less than 20%) of male sterile plants were obtained from subsequent F_1 backcross generations. The data did not fit any Mendelian genetic ratios. Cytological examination of pollen mother cells from 52 plants of these backcross populations indicated the presence of an extra chromosome in all 22 male sterile plants and the normal chromosome number (2n=38) in the remaining 30 fertile plants. Thus an extra chromosome which is derived from Diplotaxis muralis appears to cause male sterility in these backcross populations.

Introduction

Heterosis for seed yield in hybrid rapeseed (Brassica napus) has been reported by various authors (Sernyk and Stefansson 1983, Buson 1980, Guan 1980, Schuster and Michael 1976, Shiga 1976). Hybrids have shown 20-40% increase in seed production over their midparents or standard cultivars. Significant hybrid vigor in other agronomic characteristics such as plant height, leaf area, disease resistance, and lodging resistance was also observed.

Various systems have been used or proposed for the production of hybrid cultivars: treatment with male gametocides; self incompatibility mechanisms; nuclear male sterility systems, both genic and chromosomal; and cytoplasmic male sterility systems (cms). Among these, the cms system has proved to be the most effective and thus the most frequently employed method for the production of hybrid seed.

Several cms systems have been reported in species of Brassica. Ogura (1968) reported on a cytoplasmically conditioned form of male sterility in Raphanus sativus. Bannerot et al. (1977) transferred the nucleus from B. napus into the male sterile cytoplasm from R. sativus by backcrossing and established this form of cytoplasmic male sterility in B. napus. Shiga and Baba (1971) and Thompson (1972) described another cms system in B. napus.

Hinata and Konno (1979) backcrossed B. campestris into the cytoplasm from Diplotaxis muralis and reported on a cms system in B. campestris based on this cytoplasm. Shiga (1980) reported that the F_1 progeny from a cross involving B. napus and D. muralis as the female was male sterile.

To investigate the possibility of using the D. muralis cytoplasm as male sterile cytoplasm in B. napus, the B. napus cultivar Regent was crossed and then backcrossed into this cytoplasm. This paper discusses the results obtained from these crosses and demonstrates that the presence of an extra chromosome from D. muralis is the sole cause of the male sterility which is observed in these crosses.

Materials and Methods

Seeds of the sand rocket (D. muralis) were obtained from the National Institute of Agricultural Science, Japan. The sand rocket parent was crossed and backcrossed to the rape cultivar Regent (B. napus), maintaining the cytoplasm from D. muralis in all crosses. Plants with male sterile flowers were selected and used as female parents in each of six generations of backcrossing. Male sterile plants were observed in each backcross generation, but the frequency was rather low (less than 20%). Since it did not appear to be feasible to obtain a satisfactory maintainer from Regent, male sterile plants from these backcrosses were pollinated with pollen from 12 European rape cultivars. Five plants from each of the cultivars were used for crossing and the progenies were grown in the greenhouse. The numbers of male fertile and male sterile plants derived from each cross were counted and recorded.

For cytological investigation, flower buds were fixed in a Carnoy's solution consisting of 6 parts ethanol : 3 parts chloroform : 1 part glacial acetic acid. The aceto-carmine smear technique was used to examine the pollen mother cells. A total of 22 sterile and 30 fertile plants were studied cytologically at various meiotic stages. All cytological work was done on a Zeiss Photomicroscope II under phase contrast illumination. Selected cells from temporary slides were photographed with Kodak Technical Pan 2415 film.

Results and Discussion

The percentage of sterile progenies varied widely among the 12 B. napus cultivars used as the male parent, ranging from zero percent in Loras and Rabo to 36% in Karat (Table 3.1). Segregation data did not fit a one, two or three-gene model. Since the source of pollen did not provide a satisfactory genetic explanation of the results, the data were rearranged to reflect the influence of the female parent on the transmission of male sterility (Table 3.2). The frequency of steriles varied widely among different female plants, and once again no single genetic pattern could be recognized. Thus some non-Mendelian factor appeared to be involved in the expression of this male sterility.

In order to resolve the source of this male sterility, chromosome pairing was studied, using PMC's, in most cases at diakinesis. At this stage, the chromosomes are well spread and each chromosome exhibits a darkly stained centromeric region. A bivalent is seen as having two dark dots, and a quadrivalent is seen as having four. Therefore, a high degree of accuracy on the interpretations of meiotic pairing configurations can be achieved.

B. napus has a chromosome number of 2n=38 and form 19 bivalents during meiosis. In this investigation, at least 50 cells from each of the sterile plants were cytologically analyzed, and they all had 39 chromosomes which formed 19 bivalents and one univalent at diakinesis (Fig. 3.1). This univalent could easily be recognized by its smaller size and darkly stained terminal region (indicated by an arrow in Fig. 3.1).

TABLE 3.1

Influence of male parents on the F_1 progeny from backcross hybrids between male sterile plants with nuclear substitution from Regent (B. napus) into the cytoplasm of D. muralis (BC6) and 12 cultivars of B. napus.

Male Parent	Number of plants			X for 1:1 ratio	X for 3:1 ratio	X for 7:1 ratio
	Total	Fertile	Sterile			
Brutor	64	62	2	54.39*	15.19*	4.32*
Creasor	75	68	7	48.00*	9.00*	0.43
Futura	74	61	13	29.85*	1.80	1.31
Jumbo	59	46	13	17.36*	0.14	4.07*
Karat	72	46	26	5.01*	4.17*	34.57*
Kosa	72	62	10	36.13*	4.17*	0.03
Lergo	69	48	21	9.80*	0.82	18.69*
Lisandra	71	69	2	61.35*	17.47*	5.23*
Loras	67	67	0	65.01*	21.02*	8.42*
Rabo	60	60	0	58.01*	18.69*	7.47*
Sedo	63	62	1	57.14*	17.19*	5.90*
Topas	76	74	2	66.33*	19.11*	5.89*
Total	822	725	97			
Mean	69	60	10			
X for heterogeneity				30.11*	53.09*	96.02*

* P is less than 5%.

TABLE 3.2

Influence of female parents on the F_1 progeny from hybrids between male sterile plants with nuclear substitution from Regent (B. napus) into the cytoplasm of D. muralis (BC6) and 12 cultivars of B. napus

Female Parent	Total No.	Fertile		Sterile	
		Number	Percent	Number	Percent
1	59	57	97	2	3
2	51	51	100	0	0
3	55	54	98	1	2
4	59	58	98	1	2
5	53	52	98	1	2
6	83	67	81	16	19
7	99	78	79	21	21
8	38	26	68	12	32
9	30	25	83	5	17
10	30	28	93	2	7
11	49	43	88	6	12
12	56	53	95	3	5
13	53	48	91	5	9
14	56	48	86	8	14
15	51	37	73	14	27
Total	822	725		97	
Mean	55	48	88	7	12

At metaphase I, 183 out of a total of 326 cells (56%) had this univalent lying outside of the metaphase plate. As other chromosomes separated and migrated toward opposite poles, this univalent was usually left behind to form a laggard. It moved more slowly than other chromosomes but was eventually included in one of the daughter nuclei. At late anaphase I, all PMC's were found to have 19 chromosomes at one pole and 20 chromosomes at the other pole (Fig. 3.2). The second division of meiosis proceeded normally. At telophase II, two daughter nuclei had 19 chromosomes and the other two nuclei had 20 chromosomes. Very few tetrads were found to have micronuclei.

In this investigation, among the progenies from repeated backcrossing of B. napus into the D. muralis cytoplasm, all fertile plants had 38 chromosomes forming 19 bivalents during meiosis, and all sterile plants had 39 chromosomes forming 19 bivalents and one univalent. Cytological evidence seems to be strongly in favor of the theory that the male sterility is caused by the presence of the extra chromosome or genes on that chromosome. Maan (1975, 1976) reported an exceptioal case in common wheat in which all functional gametes carried an unpaired alien chromosome from Aegilops longissima or Ae. sharonensis. Apparantly, the alien chromosome in the sporophytic tissue controlled the abortion of the male and female gamet lacking the alien chromosome. In the present study, the extra chromosome seems to affect only the male gametogenesis, and not female gametogenesis because seeds were set when the male sterile flowers were pollinated with pollen grains from normal B. napus plants.

There are two possible origins of this extra chromosome. It may be an extra member of the A or C genome of B. napus or it may belong to one of the genomes of D. muralis. Since seven generations of backcrossing were performed, plants should have chromosomes mostly derived from the recurrent parent, B. napus. If the additional chromosome belongs to the A or C genome, a trivalent association would be expected in most of the PMC's. This extra chromosome also behaved differently from all other chromosomes during meiosis; it appeared as a univalent at both prophase I and metaphase I, and migrated slowly at subsequent stages. We therefore concluded that this chromosome is a member of one of the D. muralis genomes.

Presence of alien chromosomes in B. napus has also been observed by Sernyk and Stefansson (1982). A pair of Raphanus sativus chromosomes were responsible for a white flower characteristic in B. napus. Such additional alien chromosomes may be useful if they carry desirable genes.

In the present study, male sterile flowers were usually abnormal and the anthers failed to develop. However, fertile flowers with developed anthers were occasionally found on these sterile plants. The pollen grains from these anthers were examined to elucidate this phenomenon. The anthers of such fertile flowers produced pollen grains of varying size. Detailed examination of these anthers revealed pollen grains of three size classes designated as giant, normal, and small, present in numbers of 9, 346, and 383, respectively (Fig. 3.3, 3.4). Inomata (1980) observed giant pollen grains in an F_1 hybrid between B. campestris and B. oleracea. He considered these pollen grains normal in

spite of their size. Only a few giant pollen grains (9) were observed in this experiment. The small pollen grains obviously were non-functional and were considered as aborted pollen grains. The 1 : 1 (346 : 383) ratio between normal and aborted pollen grains agrees with the theoretical ratio between 'n' and 'n+1' gametes.

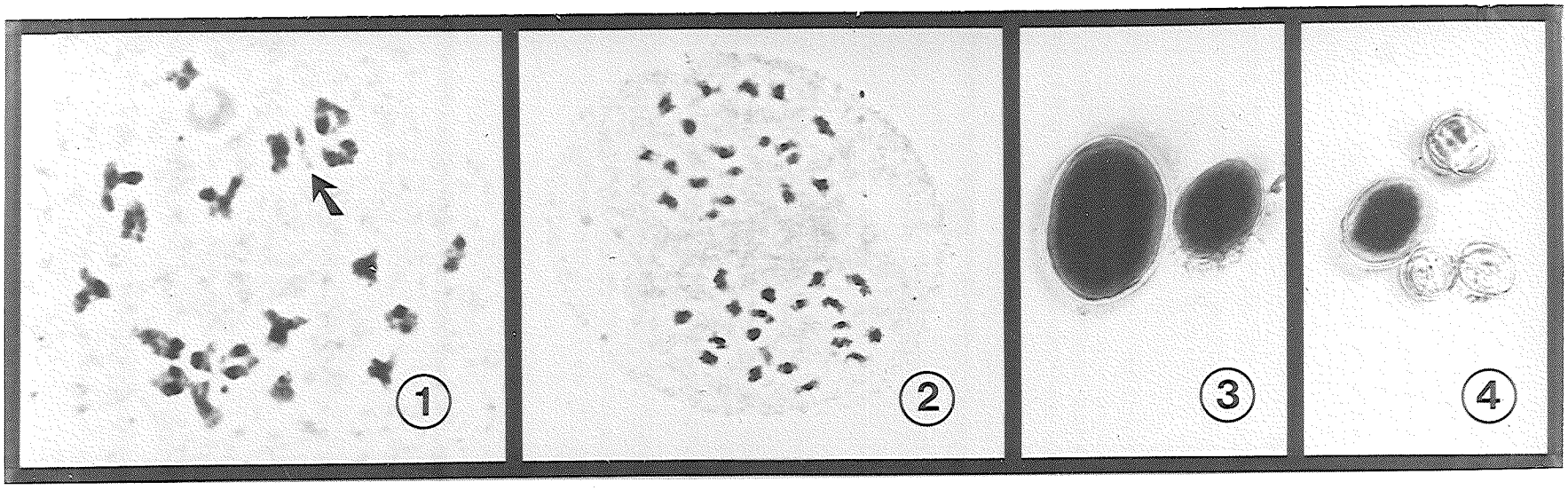
It has not been determined whether normal and aborted pollen grains carry 19 and 20 chromosomes, respectively. This could be done by pollinating normal plants with pollen grains from fertile flowers which occasionally occur on sterile plants. If the progeny all have a normal chromosome complement (2n=38), the pollen grains which were successful in making these crosses would also have carried a normal chromosome complement. Preferential transmission of normal male gametes from trisomic individuals is reported in many species (Swanson et al. 1981).

Figure 3.1: Diakinesis showing 19 II + 1 I. (The univalent is indicated by an arrow.). X 1200.

Figure 3.2: Anaphase I showing 19 chromosomes at one pole and 20 at the other. X 1200.

Figure 3.3: Pollen grains from fertile flowers on the sterile plant, from (D. muralis X Regent)(BC6) X Futura, showing giant and normal sized grains. X 450.

Figure 3.4: Pollen grains from fertile flowers on the sterile plants, from (D. muralis X Regent)(BC6) X Futura, showing normal and aborted grains. X 450.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Conclusions

The selection pressure for male sterility in backcross populations derived from D. muralis and B. napus appears to have resulted in the selection of genotypes with an extra chromosome from D. muralis. The presence of this additional chromosome from D. muralis appears to be the cause of the male sterility observed in these backcrosses. The frequency of male sterile plants (Table 3.2) apparently represent the transmission of the univalent through the female gametes. These data indicate that the average rate of transmission for the univalent is 12 percent. There was no evidence for cytoplasmic male sterility in any of the backcross progeny involving the B. napus nucleus in the D. muralis cytoplasm. This differs from the closely related species B. campestris, where the D. muralis cytoplasm does induce cytoplasmic male sterility (Hinata and Konno 1979). Thus it may be concluded that all of the Canadian and European cultivars which were tested in this study have restorers for this cytoplasm. Restoration may be associated with the C genom of B. napus because the genome as found in B. campestris does not restore fertility. (This paper has been accepted by Can. J. Genet. Cytol.)

COMPARATIVE STUDY OF FLORAL MORPHOLOGY AND STAMEN ONTOGENY OF THREE CYTOPLASMIC MALE STERILE LINES AND A NORMAL STRAIN IN RAPE (BRASSICA NAPUS L.)

Abstract

Comparative studies of floral morphology and stamen ontogeny were conducted for three cytoplasmic male sterile (cms) lines and a normal male fertile strain of Brassica napus. Morphologically, the three cms lines differed from the normal strain only in floral structure and each cms line had unique floral characteristics. The ogu cms line exhibited carpeloid and petaloid stamens. The pol cms and the nap cms lines differed in flowering habit and the relative position of anther to stigma. Histological studies showed that the stamen ontogenies of the pol and nap cms were identical. The failure of microsporogenesis was associated with the lack of differentiation of archespores. Both the pol and nap cms lines occasionally produced stamens with partially sterile anthers under the influence of high temperature. In this type of stamen, a small section of archespores differentiated and underwent normal microsporogenesis with the production of a small amount of pollen. Abnormal vacuolation in tapetal cells was found during meiosis in the ogu cms anther. The microsporogenesis of the ogu cms was inhibited at the mononucleate microspore stage with the collapse of both microspores and tapetum.

Introduction

Several cytoplasmic male sterility (cms) systems have been identified in Brassica napus. Shiga (1980) classified these cms systems based on their cytoplasmic origins and designated them as the nap, ogu, and mur cms systems respectively.

The nap cms is conditioned by the sterility-inducing cytoplasm in B. napus and was discovered in progenies from intervarietal crosses between rapeseed cultivars (Shiga and Baba 1971, 1973; Thompson 1972). The ogu cms was originally discovered in an unidentified variety of Japanese radish (Raphanus sativus L.) (Ogura 1968). This cytoplasm was later introduced into B. napus by repeated backcrossing, establishing the ogu cms in B. napus (Bannerot et al. 1977). The mur cms was developed in B. campestris by transferring the genome of B. campestris into the cytoplasm from Diplotaxis muralis (Hinata and Konno 1979). The mur cytoplasm was also used to produce male sterile plants in B. napus by means of backcrosses. The male sterility in the backcrossed progeny was associated with an extra chromosome from D. muralis (Fan et at. in press). Fu (1981) reported another cms system derived from the rape cultivar 'Polima'. The cytoplasmic origin of this cms system has not been identified. In accordance with Shiga's (1980) designation, we refer to it as the pol cms system.

Histological studies of anther development have been carried out in the nap and the ogu cms. Shiga (1976) observed that pollen mother cells (PMC) did not develop and meiosis did not occur in the sterile anther of the nap cms. Accordingly, he suggested that the inhibition of

microsporogenesis may occur between the stages of carpel differentiation III and male archesporial cell differentiation I. Ogura (1968) found that microspores in the sterile anther of the ogu cms in radish degenerated after meiosis and postulated that the abortion of microspores may be related to the early collapse of the tapetum. Bartkowiak-broda et al. (1979) reported that, in sterile anthers of the ogu cms in B. napus, there was a zone of deeply stained material which may be derived from degenerated microspores and tapetum. They suspected that the microsporogenesis was arrested at the tetrad stage but their results were not conclusive. The present study will provide detailed descriptions of floral morphology and stamen ontogeny in the male fertile control and the nap, pol and ogu cms lines, in order to identify the stage at which microsporogenesis is inhibited.

Materials and Methods

Plant materials used in this study included lines of the pol, nap, and ogu cms as well as a B. napus cultivar Regent used as the control. The pol cms line had been backcrossed with the Swedish cultivar Karat for five generations. The nap cms line was maintained for three generations by a maintainer line which had the cytoplasm from the variety Bronowski. The ogu cms line had been maintained by Regent for six generations.

Both the cms and control plants were grown in the greenhouse. Racemes with floral buds at various pre-anthesis stages of development were collected and fixed in solution of FAA. Conventional methods of dehydration and paraffin embedding were used (Berlyn and Miksche 1976).

Sections were cut at a thickness of 10-13 um and stained with iron hemotoxylin and safranin. Specimens were photographed on a Zeiss Photomicroscope II with bright field illumination. Photographs of floral morphology were taken on a Wild M-5 dissecting microscope.

Results

Floral Morphology

Regent. The floral morphology of the male fertile control is illustrated in Fig. 4.1 4.2 and 4.3. The flower was perfect and hypogynous (Fig. 4.1) and consisted of (a) a calyx with four erect sepals, (b) a corolla with four petals in the form of a cross, (c) six stamens arranged into two whorls, and (d) a gynoecium with two carpels separated by a false septum. At anthesis, the filaments of the four stamens of the inner whorl elongated so that these anthers were exposed above the receptive surface of the stigma (Fig. 4.2). The two outer stamens were distinctly less elongated and located slightly below the stigma surface. The anthers were yellow and the four loculi contained an abundance of pollen grains (Fig. 4.3).

pol cms. The flower buds of the pol cms lines usually did not open at anthesis (Fig. 4.4). Therefore, elongation of the pistil caused the stigma to protrude from the closed bud. Flowers that opened slightly or completely were also observed. The development of the stamen was severely retarded (Fig. 4.5). The filaments were very short and the anthers were reduced markedly in size and contained no pollen (Fig. 4.6). The pol cms plants also produced partially sterile anthers in

which microsporogenesis occurred only in a small part of one locule, appearing as a yellow bump (Fig. 4.7). Dehiscence of the bump was very late, probably due to a limited amount of pollen inside.

nap cms. The floral structure of the nap cms was similar to the description of Shiga (1973) of small and light colored buds, rugose petals, and short stamens with poorly developed anthers having no pollen. Distinct characteristics of the nap cms were noted. In comparison to the pol cms, the flowers of the nap cms opened normally at anthesis (Fig. 4.8). The stamen filaments were longer, thus the relative position of anther to stigma was higher (Fig. 4.9). Both sterile (Fig. 4.10) and partially sterile anthers were produced. In the latter, microsporogenesis took place in different parts of more than one locule (Fig. 4.11).

ogu cms. The floral abnormalities of the ogu cms reported by Ogura (1968) were also observed in this study (Fig. 4.12). In addition, severe modifications of stamen structure were often observed in our material. These modifications appeared to be the result of a developmental shift from the structure of the stamen towards that of the carpel or the petal. The four types of the modification were: 1) stamens with pistil-like structure (Fig. 4.13a), 2) stamens resembling a carpel with external ovules produced along edges and stigma-like structure at the distal end (Fig. 4.13b), 3) stamens with a petal-like structure (Fig. 4.13c), and 4) stamens with a sterile anther and external ovules produced near the junction of anther and filament (Fig. 4.13d). The type 2 and 4 were most commonly observed and the type 1 and 3 occurred infrequently. Every ogu cms plant produced flowers with some

form of modified stamen. The number of modified stamens varied within each flower from one to four. The two shorter stamens appeared to be less subject to these modifications.

Stamen ontogeny

Regent. The initiation of the stamen on the floral apex began with a series of divisions in the two tunica layers and the corpus. The inner tunica layer started to divide first and its periclinal divisions, together with various oblique divisions in the corpus and anticlinal divisions in the outer tunica layer, gave rise to a small projection on the floral axis. As a result of the further divisions in the tunica layers and the corpus, an undifferentiated stamen primodium with a stout and broad meristematic end was produced (Fig. 4.14). The differentiation of the stamen continued with the hyperdermal cells on both sides of the stamen primodium. These cells enlarged slightly and stained deeply with safranin (Fig. 4.15). Divisions of these cells gave rise to a few layers of derivatives which further differentiated to sporogenous cells and parietal tissue. This pattern of development indicated that the hyperdermal cells are archespores. Pollen mother cells and the anther wall were formed from the sporogenous cells and parietal tissue respectively (Fig. 4.16). The filament was derived from intercalary growth in the lower portion of the stamen. At maturity, the anther was bilobed and possessed four microsporangia.

Microsporogenesis started at an early stage of stamen development with periclinal divisions of the hyperdermal layer. This division produced the primary sporogenous cells on the inside and primary

Figure 4.1: Normal flower of Regent. X 8.

Figure 4.2: Regent flower with sepals and petals removed, showing the relative position of anther to stigma. X 10.

Figure 4.3: Normal Regent anther. X 20.

Figure 4.4: Unopened flower of pol cms. X 8.

Figure 4.5: pol flower with sepals and petals removed. X 10.

Figure 4.6: Sterile pol anther. X 20.

Figure 4.7: Partially developed pol anther. Note the swollen part of one locule which contains a small amount of pollen. X 20.

Figure 4.8: Flower of nap cms. X 8.

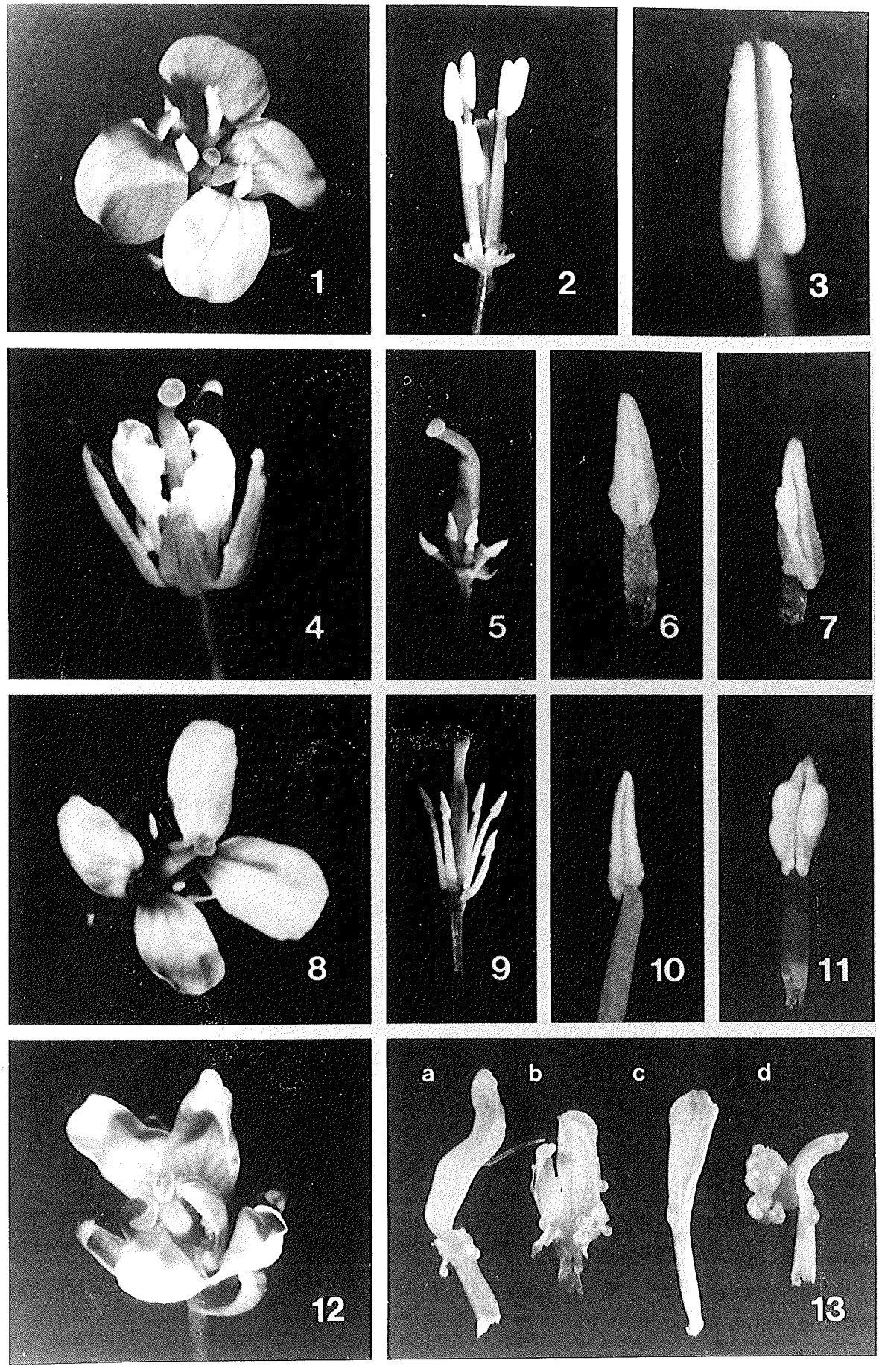
Figure 4.9: nap flower with sepals and petals removed. X 10.

Figure 4.10: Sterile nap anther. X 20.

Figure 4.11: Partially sterile nap anther. Note three swollen parts in two loculi which contain pollen. X 20.

Figure 4.12: Flower of ogu cms showing carpeloidy of anther. X 8

Figure 4.13: Structural modifications of the ogu cms anther.
a. Stamen resembling pistil. X 20.
b. Carpel-like stamen with external ovules along the edge. X 20.
c. Petal-like stamen. X 20.
d. Stamen with sterile anther and external ovules developed at the junction of the anther and the filament. X 20.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parietal layer on the outside. The parietal layer divided further to produce a wall consisting of three layers (Fig. 4.22). The innermost layer immediately adjacent to the sporogenous mass formed the tapetum. The primary sporogenous cells divided to form the pollen mother cells (PMC). Initially, the PMC's were compactly arranged and interconnected (Fig. 4.23). They later separated from each other and rounded off. At this stage, the tapetal cells became binucleate. The PMC's underwent meiosis to produce microspores (Fig. 4.24, 4.25, 4.26). During the development of the microspores, the tapetum gradually collapsed and finally disappeared completely at anthesis (Fig. 4.27).

pol cms. The earliest stages of stamen development of the pol cms were similar to that of the male fertile control. The structure of the stamen primodium initialized on the floral apex was similar and the hyperdermal cells of both the pol cms and the normal strain had dense cytoplasm and large nuclei (Fig. 4.17, Fig. 4.14). The dissimilarity in stamen development between the two genotypes was observed as stamen differentiation progressed. The derivatives from the periclinal divisions of the pol hyperdermal cells did not differentiate to sporogenous cells and parietal tissue as in the control; instead the cells resembled those of parenchyma tissue (Fig. 4.18, Fig. 4.15). Microsporogenesis was thus arrested. Such undifferentiated growth continued. Later, the hyperdermal cells on both sides of the anther and meristematic tissue at the distal end of the stamen apparently lost the ability do divide, resulting in the cessation of stamen growth. In contrast, at this stage the normal stamen had a well differentiated anther (Fig. 4.19, Fig. 4.16). The ontogeny of the pol cms stamens with

Figure 4.14: Stamen primodium of Regent. X 350.

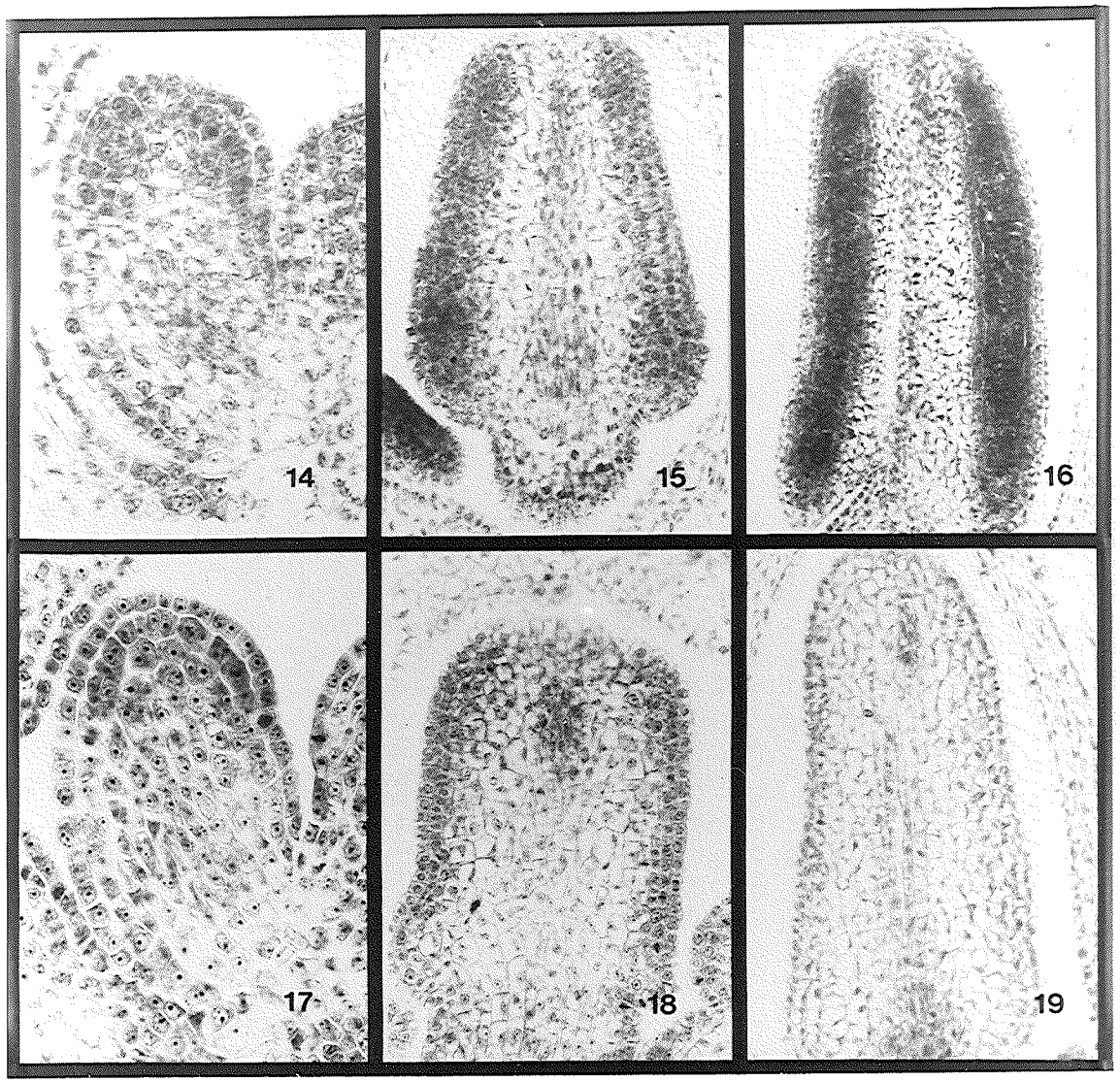
Figure 4.15: Regent stamen at the early stage of differentiation. X 470.

Figure 4.16: Regent anther at pre-meiotic stage, showing well developed wall tissue and sporogenous cells. X 210.

Figure 4.17: Stamen primodium of pol cms. X 530.

Figure 4.18: pol stamen, showing the hyperdermal cell derivatives not differentiated to sporogenous tissue. X 470.

Figure 4.19: Sterile pol anther, showing the cessation of stamen development. X 210.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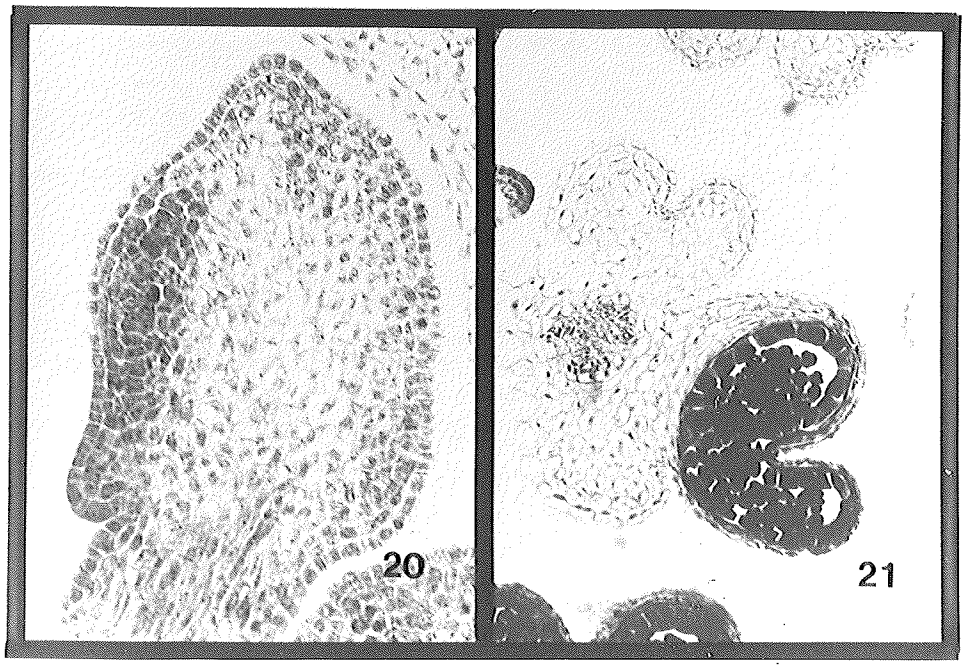
the partially developed anthers displayed the developmental features of both sterile and fertile stamens. During stamen differentiation, the hyperdermal cells appeared homogeneous, and were deeply stained. However, a small portion of the hyperdermal layer apparently undertook the function of archespores, and generated a patch of sporogenous tissue (Fig. 4.20). Microsporogenesis proceeded normally inside the patch and microspores were produced. This patch was not always located at the same position along the anther. The remainder of the hyperdermal layer gave rise to only undifferentiated tissue. In general, this limited microsporogenesis took place only in one of the four microsporangia (Fig. 4.21).

nap cms. The stamen ontogeny of the nap cms was basically identical to that of the pol cms. Sporogenous cells did not arise from the hyperdermal layer. The developmental pattern of the stamens having partially sterile anthers was also comparable to that of the pol cms. However, this limited microsporogenesis may occur in more than one microsporangium.

ogu cms. The early developmental stages of the ogu cms stamen were comparable to those of the male fertile control. Anthers of the ogu cms and the normal strain were anatomically similar, consisting of sporogenous cells surrounded by a tapetum, a middle layer, an endothecium, and an epidermis (Fig. 4.28, Fig. 4.22). However, when the meiocytes started to round off, cytoplasmic vacuoles appeared in the tapetal layer of the ogu cms anther (Fig. 4.29, Fig. 4.23).

Figure 4.20: A longitudinal section of stamen of pol cms, showing a patch of sporogenous tissue derived from a small portion of the hyperdermal layer. X 470.

Figure 4.21: A transverse section of partially sterile anther of pol cms, showing that only one locule has developed. X 380.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During prophase I, these vacuoles in the tapetal cell of the ogu cms anther increased in size and number (Fig. 4.30). Meiosis in the PMC's proceeded normally and tetrads were produced. At this stage, the tapetal cell vacuoles became quite pronounced, in contrast to dense tapetal cells of the control (Fig. 4.31, Fig. 4.25). After cytokinesis, the tapetal cells of the ogu cms anthers increased greatly in size, with the vacuoles occupying almost the entire area of the tapetal cytoplasm. The tapetal cell walls remained intact. The microspores were surrounded by exine and compacted at the center of the anther locule, possibly due to the pressure generated by the enlarged tapetum. The cellular contents of the microspores disappeared and the microspore cell walls did not form (Fig. 4.32), while the nucleoli, nucleus envelope, and surrounding exines remained visible. The tapetum and microspores later collapsed and the degenerated remnants were visible late in the floral development (Fig. 4.33). At maturity, the anther wall had lost the typical four-layer structure and became thickened. The anther locule was totally crushed. In addition, stigma-like structures at the distal end and external ovules at the base of the anther were visible.

Figure 4.22: Anther development of Regent (Fig. 4.22-27). A transverse section of anther at pre-meiotic stage. X 380.

Figure 4.23: A longitudinal section of anther at the stage that the sporogenous cells start to separate from each other. X 380.

Figure 4.24: A longitudinal section of anther at meiotic prophase. X 380.

Figure 4.25: A transverse section of anther at tetrad stage. X 380.

Figure 4.26: A longitudinal section of anther at uninucleate stage. X 210.

Figure 4.27: A longitudinal section of anther before anthesis, showing disappearance of the tapetum. X 210.

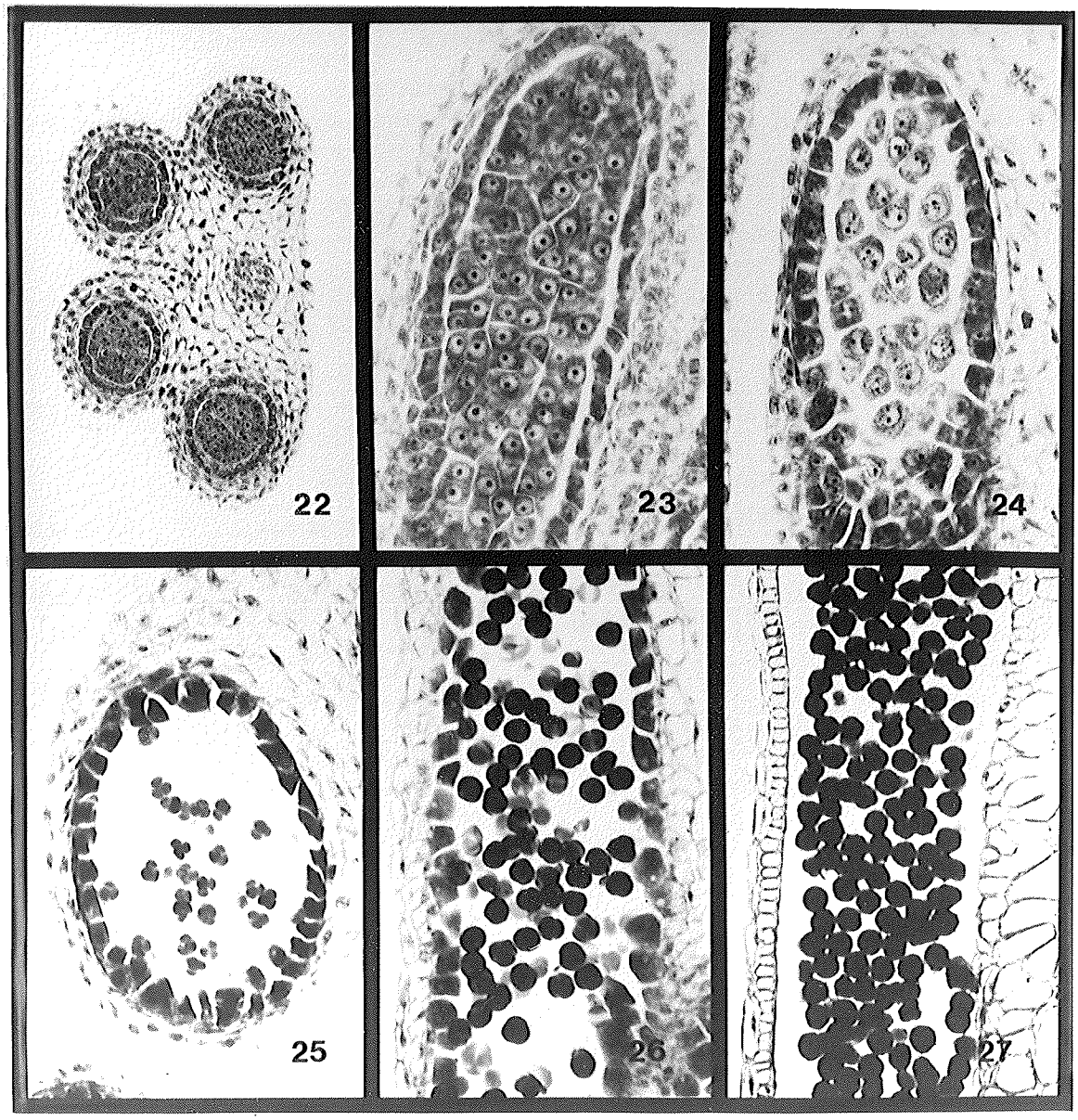
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Figure 4.28: Anther development of ogu cms (Fig. 4.28-33). A transverse section of anther at pre-meiotic stage. X 380.

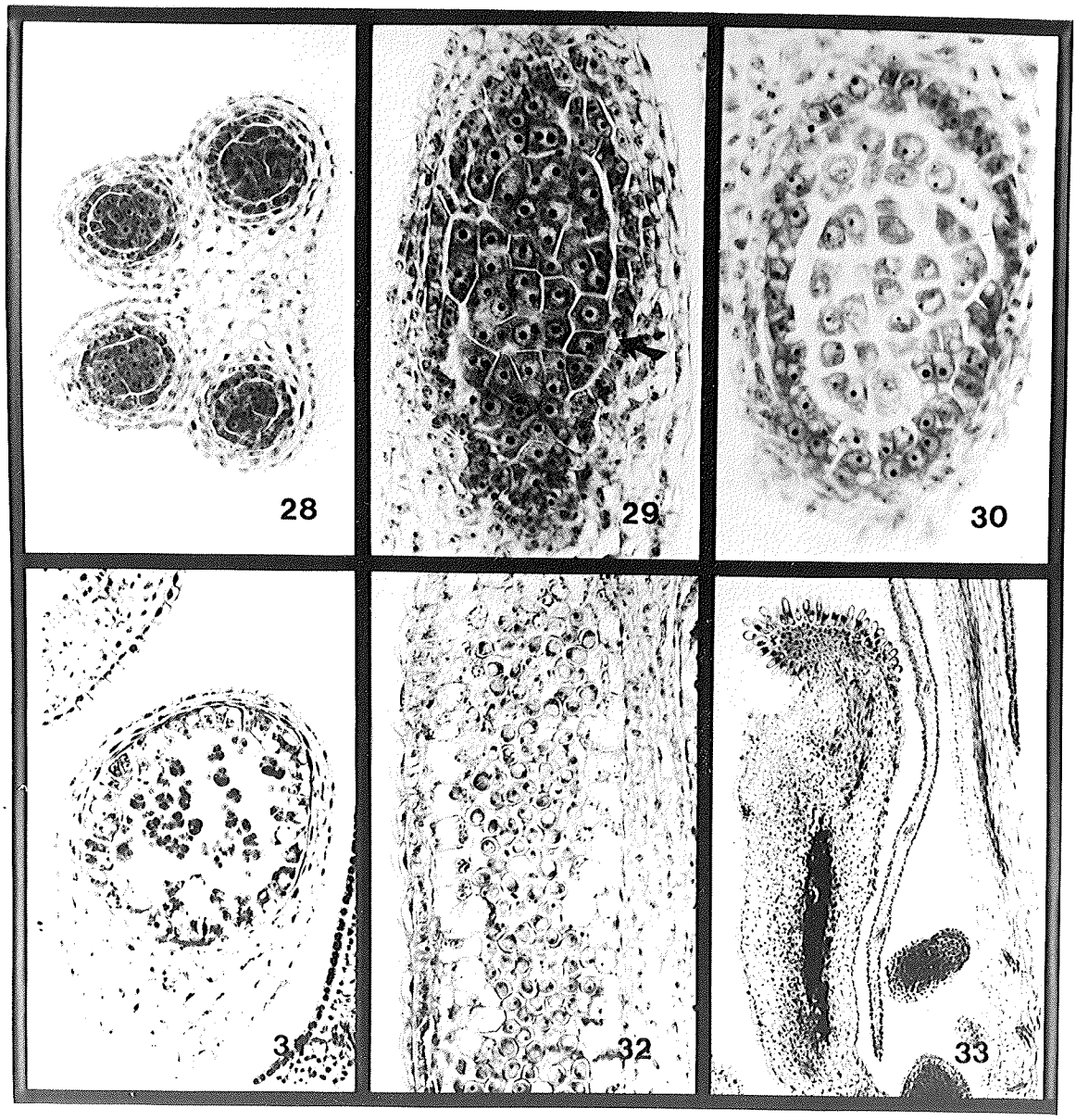
Figure 4.29: A longitudinal section of anther at the stage that the sporogenous cells start to separate from each other. Note the small vacuoles indicated by an arrow in the tapetal cells. X 380.

Figure 4.30: A transverse section of anther at meiotic prophase. Note the vacuoles have increased in size and number. X 380.

Figure 4.31: A transverse section of anther at tetrad stage, showing large vacuoles in the tapetum. X 380.

Figure 4.32: A longitudinal section of anther at uninucleate stage, showing that the tapetal cells have enlarged, the vacuoles occupy almost entire volume of the tapetal cells, and the microspores have degenerated. X 210.

Figure 4.33: A longitudinal section of anther before anthesis, showing thickening of the anther wall, the degenerated remnants of microspores and tapetum, the stigma structure, and the external ovules. X 210.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Discussion

Plants of the pol, nap, and ogu cms lines differed morphologically from the normal strain only in their floral structure. Each cms line had unique floral characteristics. The ogu cms exhibited carpeloid development of the stamens. The pol cms differed from the nap cms in flowering habit and the relative position of anther to stigma.

Although the ogu cms has been studied extensively (Ogura 1969, Bonnet 1977, Bannerot et al. 1977, Heyn 1978), carpeloidy of the stamens had not been reported in this cms system. The ogu cms line used in this study had been backcrossed for seven generations to the spring rape cultivar Regent and showed both carpeloid and petaloid stamens. This line had also been crossed with a number of spring rape cultivars to test for fertility restoration. Some of these F_1 hybrids expressed the carpeloid development. This information suggests that the carpeloidy arises from an interaction between the ogu cytoplasm and certain nuclear gene(s). Pearson (1972) reported a very similar situation in B. oleracea. A cms system of B. oleracea with the exotic cytoplasm from B. nigra expressed a petaloid sterility in which stamens were transformed to petals and carpels with the absence of nectaries. His published illustrations showing the stamen morphology of such flowers are very similar to those of the ogu cms flowers. Pearson (1972) also indicated that the petaloid sterility was conditioned by a single recessive gene(p).

The early developmental stages of stamen ontogeny in the pol cms and in the normal strain were similar in the pattern of stamen initiation on

the floral apex and the structures of stamen primodia. The divergent development of the pol cms stamen was observed during stamen differentiation. Failure of archesporial differentiation in the hyperdermal layer apparently resulted in the sterility of the pol cms system. In the stamens with partially sterile anthers, a small portion of the hyperdermal cells differentiated into archespores. Microsporogenesis continued, ending in the production of microspores. Those hyperdermal cells which did not produce archespores gave rise to only undifferentiated parenchyma-like tissue. This developmental difference is influenced by temperature. Fu (1981) reported that the pol cytoplasmic male sterility was unstable and broken down under high temperatures. It appears that treatment with high temperature acts to either promote or inhibit certain substances, probably plant hormones, which in turn trigger the differentiation of the hyperdermal cells into archespores.

The inhibition of microsporogenesis in the nap cms was associated with failure of archesporial differentiation. This observation is consistent with the suggestion made by Shiga (1976). Even though the nap and the pol cms systems exhibited similar patterns of stamen development, they differed markedly in the pattern of fertility restoration. Thompson (1972) and Shiga (1976) reported that most rape cultivars tested were effective restorers and partial restorers for the nap cms system and a few cultivars were maintainers. This situation is also observed in our breeding program. However, the fertility restorers for the nap cms are usually sterility maintainers for the pol cms. None of the rape cultivars tested in our program were able to effectively

restore the fertility of the pol cms. This implies that the sterilities of the two cms systems may be conditioned by different genetic factors although the two systems have similar stamen development.

The microsporogenesis of the ogu cms has been studied by Ogura (1968) and Bartkowiak-Broda et al. (1979). They showed that pollen degeneration occurred at the microspore stage and suggested that the abortion of microspores may be associated with the abnormal behavior of the tapetum. The results of the present study provides additional evidence to support this suggestion. The striking feature of the tapetal abnormality in the ogu cms system is the vacuolation which appeared at the stage of sporogenous mass separation. Meiosis was apparently not affected by the abnormal tapetum. The collapse of the microspores and the tapetum occurred at the uninucleate microspore stage.

Aberrant vacuolation of tapetal layer has been reported in cytoplasmic male sterility in Sorghum (Overman and Warmke 1972), Capsicum (Horner and Rogers 1974), Helianthus (Horner 1977), and Secale (Scoles and Evans 1979). This abnormal behavior of the tapetum has been held responsible for the induction of male sterility (Edwardson 1970, Laser and Lersten 1972). As the tapetal layer is the tissue in the closest contact with reproductive cells, it may play an essential nutritive role during microsporogenesis (Mascarenhas 1975). The tapetum has been suggested as the source of callase which dissolves the callose wall and releases the microspores from tetrads (Izhar and Frankel 1971, Stiegitz 1977). In some plant species, the tapetal cells also influence the pollen wall maturation and triphine deposition (Heslop-Harrison and

Dickinson 1969, Horner and Pearson 1978). Therefore, any disturbance that prevents the tapetum from performing such functions might result in male sterility.

INFLUENCE OF TEMPERATURE ON STERILITY OF TWO CYTOPLASMIC MALE STERILITY SYSTEMS IN RAPE (BRASSICA NAPUS L.)

Abstract

The effect of temperature on two cytoplasmic male sterility (cms) systems in rape (Brassica napus L.) was investigated. These were the nap cms system with the cytoplasm which occurs in most Canadian cultivars and the pol cms system with the cytoplasm from the cultivar Polima. The day/night temperature regimes used were 22/16, 26/20, and 30/24° C. Two floral characteristics, anther type and stamen length, were influenced by temperature treatments. Male sterility of both cms systems was expressed consistently at the lowest temperature. The nap male sterile plants became partially sterile at the second temperature and fully fertile at the highest temperature. The pol male sterile plants were more stable and became partially sterile only at the highest temperature.

Introduction

Interest in the development of hybrid cultivars in rape (Brassica napus) was stimulated by the discovery of cytoplasmic male sterility (cms). Several cms systems have been identified . Shiga and Baba (1971, 1973) and Thompson (1972) described a cms system conditioned by the sterility-inducing cytoplasm of B. napus, designated as the nap cms system (Shiga 1980). Bannerot et al. (1977) developed a cms system by

backcrossing the genome of B. napus into the male sterile cytoplasm of radish (Raphanus sativus) discovered by Ogura (1968). Shiga (1980) classified it as the ogu cms system. Fu (1981) reported another cms system which occurred naturally in the rape cultivar, Polima, obtained from Poland. We designated it as the pol cms system.

The expression of male sterility in both the pol and the nap cytoplasms is influenced by environmental conditions. Shiga (1973) reported that the nap cms plants may produce functional pollen at the late stage of flowering depending upon climatic conditions, especially air temperature. Fu (1981) observed a similar phenomenon with the pol cytoplasm; plants became fertile under high temperatures in the field. This response to high temperature complicates genetic studies of fertility restoration and selection of sterility maintainers for both cms systems.

It is essential to have more precise information on the effect of temperature on male sterility in order to utilize the pol and the nap cms systems in production of commercial hybrids. This paper reports results of investigations on the influence of temperature on the expression of sterility in the pol and the nap cms systems.

Materials and Methods

Sterile and partially sterile lines of the pol cms and the nap cms systems, and a rape cultivar Regent were used in this investigation. The sterile plants were characterized by undeveloped anthers and short stamen length (Fig. 5.1A for the pol steriles; Fig. 5.2A for the nap

steriles). The partially sterile plants were identified by poorly developed anthers containing a very limited amount of pollen, and stamen length which was longer than that of the sterile anthers (Fig. 5.1B for the pol partially steriles; Fig. 5.2B for the nap partial steriles).

The sterile and partially sterile lines of the pol cms had been maintained by the cultivars Karat and Regent for five and six generations, respectively. The maintainer for the nap cms system was developed by using the infrequently formed pollen grains from the male sterile plants to pollinate plants with the cytoplasm from the cultivar Bronowski. The partially sterile lines of the nap cms were propagated by self-pollination.

Sterile and partially sterile plants of both cms systems and fertile plants of Regent were grown in flats filled with a mixture of soil, sand, and peat (2 : 1 : 1). In previous breeding experiments, we observed that the cms plants were not sensitive to high temperature during the vegetative stage but were sensitive during the reproductive stage. Therefore, plants were grown in the greenhouse until emergence of the racemes was complete. They were then transferred to growth rooms at day/night temperature regimes of 30/24, 26/20, and 22/16° C for seven days. After these treatments, the plants were transferred to the growth room at 22/16° C. The photoperiod was 16 hours. Photosynthetically active radiation (PAR) was provided by VHO (very high output) Gro-Lux WS (wide spectrum) fluorescent lamps. Photosynthetic photon flux density (PPFD) was 250 uE m^{-2} s^{-1} measured at the top of the plants. Ten plants from each of the experimental lines were used for each temperature treatment. The test was replicated four times.

Two floral characteristics influenced by temperature, anther type and stamen length, were used to measure the effect of temperature on the expression of male sterility for both the pol and the nap cms systems. Flowers were classified into three types based on the level of anther development: male sterile, partially male sterile, and male fertile. Eight measurements for stamen lengths were taken for each cms line in each replicate. Analysis of variance was used to evaluate the data on the stamen length.

Result

All plants began to bloom 2-4 days after transfer to the growth room at 22/16° C. Observations on stamen development and pollen production were began soon after flowering. The plants were observed at intervals of two days for about 40 days.

Sterile and partially sterile plants of the pol cms grown at the temperature regime of 22/16° C remained stable. The sterile plants produced only sterile anthers with no pollen (Fig. 5.3A), while the partially sterile plants produced anthers containing minute quantities of pollen (Fig. 5.3B). Sterile and partially sterile plants of the nap cms reacted in a similar manner (Fig. 5.4A, B.). Since expression of male sterility of these plants was not altered at temperature of 22/16° C, sterile and partially sterile plants of the two cms systems grown at this temperature regime were used as experimental controls. The fertility of Regent was not influenced by any temperature treatment.

After treatments at higher temperature regimes (26/20 and 30/24° C), the sterile and partially sterile plants of the pol and the nap cms systems displayed a distinct pattern of fertility expression in response to temperature. During the first 8-10 days of flowering, the plants did not show any change in stamen morphology when compared with the control plants. After this period, increasing fertility of the newly opened flowers was manifested by altered development of anthers (Table 5.1) and elongation of stamen (Table 5.3). The production of flowers with improved fertility continued for about 8 to 10 days. The flowers which opened after this period expressed the original sterile and partially sterile status for the remainder of this study.

The data on anther development of male sterile and partially male sterile plants treated at different temperature regimes are summarized in Table 5.1. Sterile and partially sterile plants of the pol cms treated at 26/20° C were similar to the control plants in anther type. Apparently, sterility expression of these two lines was not influenced by this temperature. However, changes in stamen development were observed on both lines of the nap cms. Partially sterile flowers appeared on the sterile plants and fertile flowers (Fig. 5.4C) on the partially sterile plants. At the temperature regime of 30/24° C, the stamen development of all plants of the two cms systems was affected. Expression of the pol sterility was altered only by the highest temperature. Fertile anthers produced on the partially sterile plants of the pol cms is illustrated by Fig. 5.3C. Both lines of the nap cms were male fertile at 30/24° C. Although the fertile flowers produced after high temperature treatments had anthers possessing four developed

TABLE 5.1

The effect of temperature on anther type in sterile and partially sterile lines of the pol and the nap cms in Brassica napus.

cms line	Temperature regimes (day/night° C)* 22/16	26/20	30/24
pol sterile	S	S	PS
pol partially sterile	PS	PS	F
nap sterile	S	PS	F
nap partially sterile	PS	F	F

* S - sterile; PS - partially sterile; F - fertile;

loculi with an abundance of pollen, they were somewhat abnormal with narrower petals and shorter stamens than those of the maintainers (Fig. 5.3D; Fig. 5.4D).

The analysis of variance on stamen length (Table 5.2), which showed significant values for the main factors and all interactions between them, indicates the importance of the effect of temperature on stamen length. Stamen length for both nap and pol cms plants increased as temperature increased (Table 5.3). Shiga (1976) used the relative position of the anther to the stigma to predict the levels of sterility for the nap cms plants. This relationship between stamen length and anther development can be observed in Figs. 1, 2, 3, and 4.

Figure 5.1: pol cms flowers (petals removed).
A. male sterile.
B. partially male sterile.
C. male fertile produced under high temperature (30/24° C).
D. maintainer for the pol cms, Regent.

Figure 5.2: nap cms flowers (petals removed).
A. male sterile.
B. partially male sterile.
C. male fertile produced under high temperature (30/24° C).
D. maintainer for the nap cms.

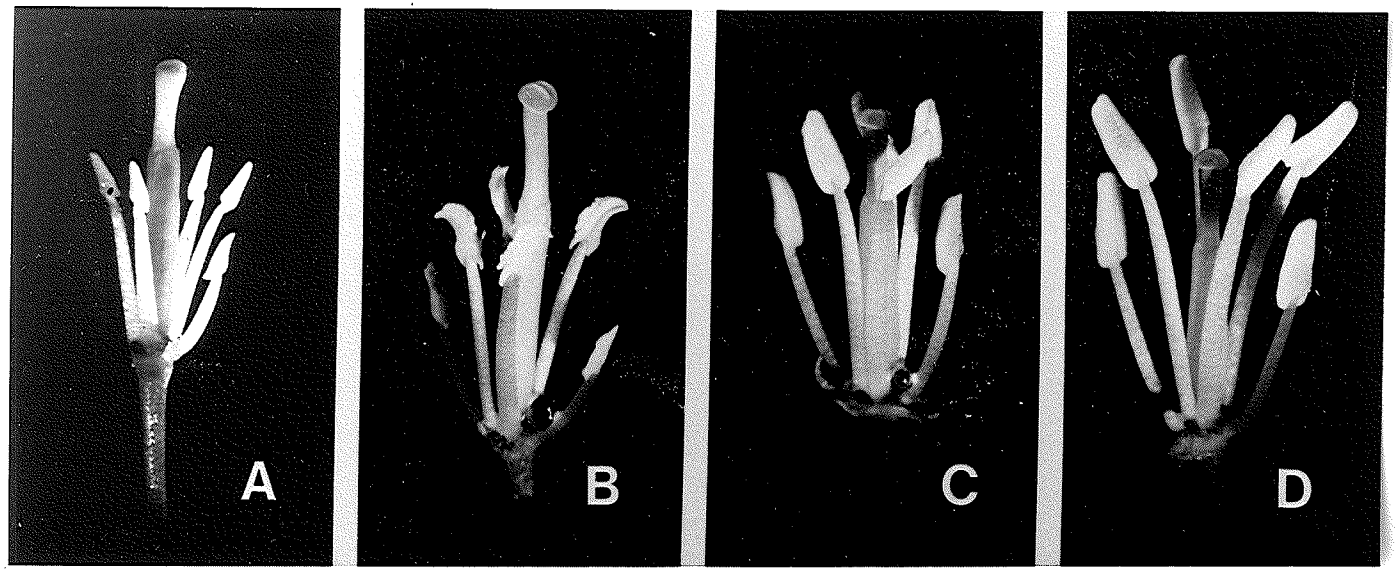

A
B
C
D

Figure 5.3: pol cms anthers.
A. male sterile.
B. partially male sterile.
C. male fertile produced under high temperature (30/24° C).
D. maintainer for the pol cms, Regent.

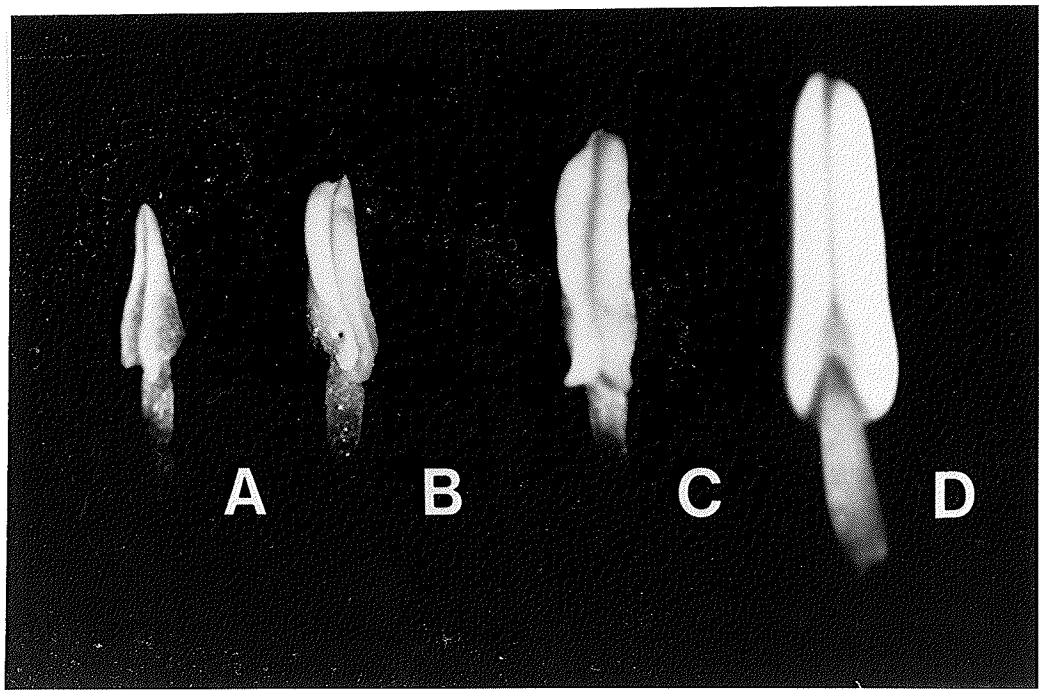

A
B
C
D

Figure 5.4: nap cms anthers.
A. male sterile.
B. partially male sterile.
C. male fertile produced under high temperature (30/24° C).
D. maintainer for the nap cms.

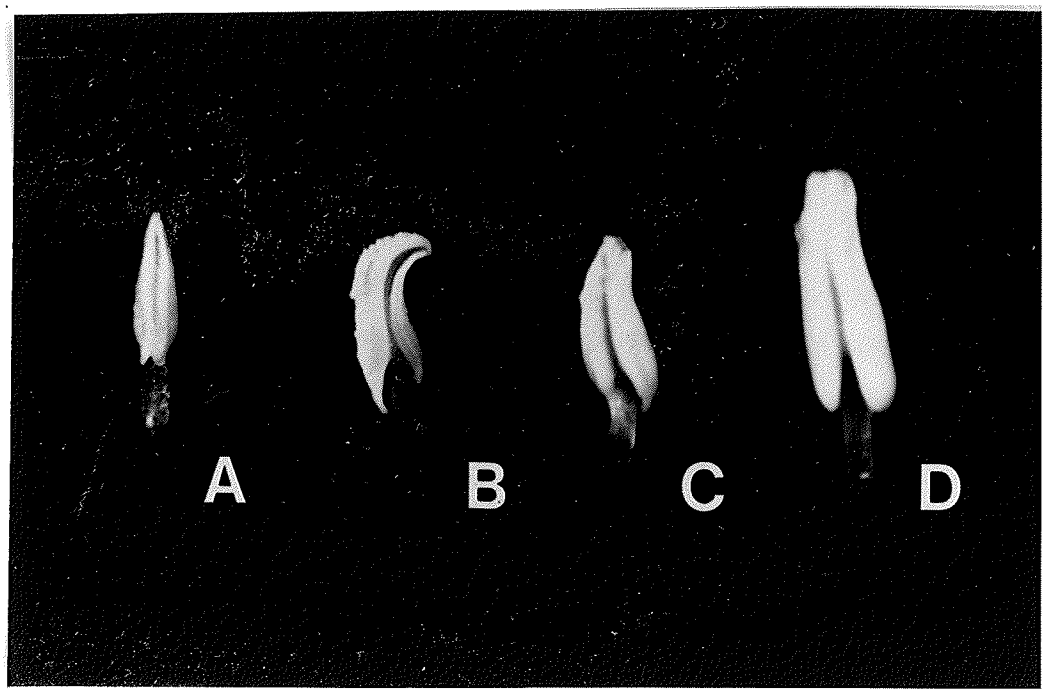

A
B
C
D

TABLE 5.2

Analyses of variance for stamen length of sterile and partially sterile lines of the pol and the nap cms systems treated at three different temperatures.

Source of variation	df	Sum of squares	Mean square	F value
Temperature (Temp)	2	270.63	135.32	199.58*
cms system (cms)	1	1211.26	1211.26	1786.52*
Temp X cms	2	168.35	84.18	124.15*
Sterility level (level)**	2	283.75	141.38	208.52*
Temp X level	4	25.56	6.39	9.43*
Replication	3	0.72	0.24	0.36
Error	369	250.18	0.68	

* Significant at 0.01 probability level.
** Sterility level is nested in cms system.

TABLE 5.3

The effect of temperature on stamen length (mm) in sterile and partially sterile lines of the pol and the nap cms in Brassica napus.

cms line	Temperature regimes (day/night° C) 22/16	26/20	30/24
pol sterile	3.66a*	3.56a	4.36b
pol partially sterile	5.63a	5.56a	6.39b
nap sterile	6.16a	8.47b	8.55b
nap partially sterile	6.53a	10.13b	10.64b

* Values followed by the same letter within each cms line are not statistically different at 0.05 probability level using the least significant difference test after the analyses of variance.

Discussion

This study indicates that temperature plays an important role in the expression of male sterility in lines carrying the pol and the nap cms cytoplasms. High temperature promotes better development of anthers and increases stamen length, whereas low temperature ensures consistent expression of the male sterility. The effects of environmental conditions on cytoplasmic and genic male sterilities have been investigated in corn (Duvick 1965), cotton (Sarvella 1966, Meyer 1969), Petunia (Marrewijk 1969), broccoli (Dickson 1970), soybean (Stelly and Palmer 1980), and common bean (Estrada and Mutschler 1984). All investigations reveal that temperature is the major environmental factor that interacts strongly with fertility restoring mechanisms in male sterile plants, although photoperiod may also affect male sterility.

Both the pol and nap cms plants were sterile at the lowest temperature (22/16° C) but the temperatures required to stimulate anther development for the two types of cms were different. A temperature regime of 30/24° C was required to convert the anther of the pol cms plants from sterile to partially sterile, while 26/20° C was sufficient to achieve the same conversion for the nap cms plants. Thus, the pol cms is more stable than the nap cms and plants with the pol cytoplasm have a higher degree of sterility than those with the nap cytoplasm.

A time interval of 10 to 14 days between temperature treatments and plant response was observed for both the pol and the nap cms systems. A similar phenomenon was also reported for cytoplasmic male sterility in cotton (Narshall et al. 1974) and Petunia (Izhar 1975). We have

observed that on fertile plants of the cultivar Regent, floral buds less than 1 mm in length usually take 12 to 16 days to flower in the summer greenhouse. Histologically, such floral buds are at developmental stages prior to stamen differentiation. Studies of stamen ontogeny of the pol and the nap cms revealed that the two cms system had the same pattern of stamen development and that sterility was associated with failure of differentiation of archespores. Thus, temperature must operate on buds at stages before archesporial differentiation to promote normal development of the stamens.

The sensitivity of the pol and the nap cms plants to temperature may reduce their utility for the development of hybrid cultivars. However, the short stamens, late dehiscence and limited amount of pollen associated with partial sterility are all unfavorable for self-pollination. Furthermore, mixtures of up to 50% of seed from one parent with hybrid seed may produce seed yields essentially equal to 100% hybrids under dense planting (Murakami et al. 1969, Shiga 1970). The variation in degree of sterility in both the nap and pol male steriles under the higher temperatures also suggests the possibility of selecting maintainers which would produce more stable male steriles. (This paper has been submitted to Can. J. Plant Sci.)

MAINTAINERS AND RESTORERS FOR THREE MALE STERILITY INDUCING CYTOPLASMS IN RAPE (BRASSICA NAPUS L.)

Abstract

The F_1 progenies from crosses involving 32 cultivars and/or lines and male sterile plants with each of the three cytoplasms, ogu, nap, and pol were evaluated for male fertility. All strains were maintainers for the ogu cytoplasm. The fertility of the nap male sterile plants was fully restored by 30 strains. Bronowski partially maintained the nap male sterility and segregation was observed in the F_1 hybrids involving the nap male sterile plants and Lergo. Thus Lergo appears to be heterogeneous for genes conditioning maintenance and restoration of this type of male sterility. None of the strains restored the pol male sterility effectively.

The F_2 and backcross data obtained under a controlled environment suggests that both Karat and Westar possess a single dominant gene for the restoration of fertility in the nap male sterile plants.

Fertility of the pol male sterile plants was restored in the F_1 of crosses involving the strain of Brassica juncea known as Zem. The fertile interspecific hybrid was backcrossed to Regent four times and resulting plants were self-polloinated for three generations. Five male fertile lines which had the pol cytoplasm were obtained. The chromosome numbers of single plants from these population ranged from 37 to 40. Apparently selection for fertility during backcrossing and

self-pollination may have resulted in the selection for a chromosome involved with restoration from B. juncea.

Introduction

Substantial heterosis has been reported in first generation hybrids of rape (Brassica napus L.) (Sernyk and Stefansson 1983, Hutcheson et al. 1981, Morice 1978, Shiga 1976, Schuster and Micheal 1976). The seed production from F_1 hybrids may exceed that of the parents by 20 to 40%. An effective mechanism of pollination control is required so that this potential for higher yield can be exploited on a commercial scale. The first reports of cytoplasmic male sterility (cms) in rape (Shiga and Baba 1971, Thompson 1972) stimulated interest in the development of hybrid rape cultivars.

In rape, several cytoplasmic male sterility inducing cytoplasms are available. Ogura (1968) discovered male sterility in the radish (Raphanus sativus). The nucleus of B. napus was transferred into this cytoplasm using interspecific crosses and subsequent backcrosses (Bannerot et al. 1977). Several backcrosses were made to produce cms lines with the radish cytoplasm and the nuclei from certain cultivars. Shiga and Baba (1971, 1973) and Thompson (1972) discovered male sterile plants in segregating populations from certain crosses and established that the male sterility was conditioned by a male sterile cytoplasm which occurred in B. napus. Shiga (1980) designated the sterility inducing cytoplasms from R. sativus and B. napus as ogu and nap, respectively. Fu (1981) reported on male sterile plants which occurred spontaneously in plants grown from seed of the cultivar Polima. This male sterility inducing cytoplasm is designated as pol in this paper.

Fertility restoration for the ogu and nap cms has been investigated. Heyn (1979) and Rousselle (1979) found that all rape cultivars tested in their studies were maintainers for the ogu cms. Bonnet (1975) reported that some European radish cultivars were able to restore the fertility of the ogu cms. Therefore, an attempt was made to transfer the fertility restoring factors from radish to B. napus (Heyn 1979). The nap cms is restored by many rape cultivars but only a few varieties are able to maintain the sterility (Thompson 1972, Shiga 1976, Shiga et al. 1983). Genetic studies indicated that the fertility restoration for the nap cms was controlled by 1 to 5 dominant genes depending upon the cultivar tested (Thompson 1972, Shiga 1976, Shiga et al. 1983, Rousselle and Renard 1982, Sernyk 1982). Little information appears to be available concerning maintenance of sterility and restoration of fertility for the pol cytoplasm. This paper reports on the ability of 22 strains to act as maintainers or restorers for the three male sterility inducing cytoplasms, the inheritance of restoration for two cultivars using the nap cytoplasm, and the transfer of a fertility restoring factor for the pol cytoplasm from B. juncea.

Materials and Methods

Three male sterility inducing cytoplasms, ogu, nap, and pol were evaluated. Male sterility in the ogu cms line had been maintained by the cultivar Regent for six generations. The nap male sterile plants were maintained using functional pollen which is produced when these male sterile plants are grown under a high temperature. Male sterility in the pol cytoplasm had been maintained by the cultivar Karat for five generations.

Thirty-two strains from 9 countries were chosen and used as pollen parents in crosses to each of the three male sterile cytoplasms (Table 6.1). The strains from China and Japan were winter rape. The seedlings from these strains were vernalized at 4° C for 30 days to induce flowering. Sixty F_1 plants from each cross were grown in the greenhouse and levels of male fertility of these plants were evaluated. The male fertility was classified into three types, male sterile, partially male sterile, and male fertile, according to the degree of anther development. Male sterile plants were identified by undeveloped anthers having no pollen. Partially male sterile plants produced anthers containing a small amount of pollen. Male fertile plants had fully developed anthers with an abundance of pollen.

Karat and Westar were used as pollen parents in crosses with the nap male sterile plants to obtain information about inheritance of the male sterility. The sterile line had been maintained for three generations using a maintainer line with the cytoplasm from Bronowski. The F_1 plants were grown in the greenhouse and selfed to produce F_2 seeds. The backcrossed generation was produced by pollinating the male sterile plants with pollen from the F_1 plants. The F_2 and backcrossed progenies were initially grown in the greenhouse. One week after bolting, the plants were transferred to a growth room at temperature of 22/16° C (day/night). Sixteen hours of illumination were provided using VHO (very high output) Gro-lux (wide spectrum) fluorescence lamps. Data on male fertility and sterility were collected. Chi-Square tests were used to test the fit of observed to expected genetic ratios and heterogeneity chi-squares were calculated using data of four F_2 and backcrossed families.

Since most cultivars were maintainers or partial maintainers for the pol sterility inducing cytoplasm, pollen from a strain of Brassica juncea known as Zem was used to pollinate male sterile plants with the pol cytoplasm. The F_1 hybrids produced normal anthers and were fertile. Several backcrosses were made using Regent as the pollen parent and fertile segregants as the female parents. After the third backcross, fertile plants were self-pollinated for three generations, five lines which appeared to be uniformly self-fertile in the pol cytoplasm were selected. To study the inheritance of fertility restoration, one plant from each of these five fertile lines was used to pollinate male sterile plants with the pol cytoplasm. Twenty F_1 plants from each cross were grown in the greenhouse and male fertility of these plants was recorded.

In order to determine whether these five fertile lines had obtained a normal chromosome complement (2n=38), flower buds from 35 fertile plants were collected and fixed in a "Carnoy" solution consisting of six parts of absolute alcohol, three parts of chloroform, and one part of glacial acetic acid (v:v:v). Anthers were smeared with aceto-carmine. More than 50 pollen mother cells at diakinesis from each plant were examined under a Zeiss Photomicroscope II with phase contrast illumination.

Results and Discussion

The F_1 plants from crosses between the 32 strains and male sterile plants with the ogu cytoplasm were all completely male sterile. Similar results have been reported by Heyn (1979) and Rousselle (1979). All cultivars of B. napus that have been tested are maintainers for the ogu cytoplasm. Some European radish cultivars apparently possess fertility

restoration genes for the ogu cytoplasm (Bonnet 1975), thus, utilization of the cytoplasm apparently depends on the degree of success which can be achieved in introgressing these genes from Raphanus into B. napus.

Thirty of the 32 progenies from crosses involving male sterile plants with the nap cytoplasm and pollen from the strains listed in Table 6.1 were fully fertile. The anthers of the F_1 progenies from the cross involving Bronowski as the pollen parent contained a small amount of pollen. Bronowski has been reported to have the fertile cytoplasm of B. napus and recessive gene rf rf and the nap male sterility maintained by Bronowski was not complete (Thompson 1972). The F_1 plants involving Lergo as a pollen parent segregated, producing fertile, partially sterile and sterile plants. Thus, Lergo appears to be heterogeneous for restorer gene(s). Only a few cultivars have been identified as maintainers for the nap cytoplasm (Thompson 1972, Shiga 1976, Shiga et al. 1983) and these cultivars do not have canola quality (low erucic acid content in the oil and low glucosinolate content in the seed). The oil and seed from Lergo meet the requirements for canola quality. The segregants from Lergo may be used to facilitate the development of a canola quality maintainer for the nap cytoplasm.

None of the F_1 plants from crosses involving male sterile plants with the pol cytoplasm and 32 pollen parents (Table 6.1) were completely fertile. The progenies from most of the crosses were male sterile. However, nine of the crosses produced partially sterile F_1 hybrids (Table 6.1). Both the nap and pol cytoplasms are derived from cultivars of B. napus. However, most strains are restorers for the nap cytoplasm, but are maintainers or partial maintainers for the pol cytoplasm. Thus,

TABLE 6.1

The male fertility or sterility of F_1 hybrids from crosses involving male sterile plants from three male sterile cytoplasms and thirty-two strains from seven countries.

Cultivar or Strain	Country of Origin	Reaction in Sterility Inducing Cytoplasm* ogu	nap	pol
Bronowski	Poland	S	PS	S
Creasor	France	S	F	PS
Orpal	France	S	F	S
R83-5	France	S	F	S
R83-9	France	S	F	S
R83-14	France	S	F	S
Sedo	Germany	S	F	PS
Prota	Germany	S	F	S
Futura	Germany	S	F	PS
Loras	Germany	S	F	S
Jumbo	Germany	S	F	S
Lisandra	Germany	S	F	PS
Kosa	Germany	S	F	PS
Rabo	Germany	S	F	S
Nicklas	Sweden	S	F	S
Karat	Sweden	S	F	S
Topas	Sweden	S	F	PS
Lergo	Sweden	S	Seg.	S
Regent	Canada	S	F	PS
Westar	Canada	S	F	PS
Erhao	China	S	F	S
Sihao	China	S	F	S
4581-1	China	S	F	S
5339-1	China	S	F	PS
2338-1	China	S	F	S
Norin 10	Japan	S	F	S
Norin 16	Japan	S	F	S
Chisaya-Natane	Japan	S	F	S
Asahi-Natane	Japan	S	F	S
111	India	S	F	S
129	India	S	F	S
123	India	S	F	S

* S - sterile; P - partially sterile;
F - fertile; Seg. - segregation;

the two sterility inducing cytoplasms are conditioned by different genetic mechanisms.

Since a fertility restorer for the ogu and pol cytoplasms was not available, the genetic study of fertility restoration was limited to the nap cytoplasm. The F_2 generation involving Karat as pollen parent segregated with 231 fertile and 66 sterile plants, and the corresponding backcross produced 73 fertile and 54 sterile plants (Table 6.2). Chi-square tests indicate a satisfactory fit of these observed numbers to 3 : 1 and 1 : 1 ratios, respectively, and all chi-squares for heterogeneity were not significant. Thus, fertility restoration in this case appears to be conditioned by a dominant allele at a single locus. In the cross involving Westar as the pollen parent, segregation in the F_2 generation was 179 fertile and 23 sterile plants, and that of the corresponding backcross was 47 fertile and 50 sterile plants (Table 6.2). This F_2 data do not fit either a 3 : 1 or a 15 : 1 ratio, while the backcross data were in agreement with a 1 : 1 ratio. While segregation at a single locus seems most plausible, other explanations such as heterogeneity for restorers within the variety cannot be eliminated on the basis of the data which is available.

In a similar experiment, Thompson (1972) also reported that one dominant gene conditioned the fertility restoration. More complex inheritance also occurs. Shiga (1976) and Shiga et al. (1983) indicated that the restoration was controlled by dominant genes at 2 to 4 loci, depending upon cultivars used. Sernyk (1982) suggested an even larger number of genetic factors which influence the restoration.

TABLE 6.2

Segregation for male sterility and male fertility in the F_2 and backcrossed generations involving the nap cms plants and pollen parents Karat and Westar.

Generation	No. of plants	Fertile	Sterile	Ratio	X
(nap) cms X Karat (F2)	297	231	66	3 : 1	1.08
		Heterogeneity			3.30
(nap) cms X Karat (BC)	127	73	54	1 : 1	2.55
		Heterogeneity			0.37
(nap) cms X Westar(F2)	202	179	23	3 : 1	19.25*
				15 : 1	8.24*
(nap) cms X Westar(BC)	97	47	50	1 : 1	0.04
		Heterogeneity			0.09

* indicates that the segregation data do not fit the proposed model significantly at p=0.05.

These reports indicate that cultivars may possess one to several genes for restoring fertility of male sterile plants with the nap cytoplasm. However, high temperatures are known to influence the expression of sterility in the nap cytoplasm (Thompson 1972, Shiga 1976). Sterile plants are converted to partially sterile and fully fertile after treatments at high temperatures (26/20° C and 30/24 C, day/night) for seven days, while the sterility is maintained at lower temperature (22/16° C, day/night). Thus, if the populations segregating for male sterility are not grown under controlled temperature, there will be a tendency to overestimate the number of genes involved.

Since an effective restorer for the pol cytoplasm was not available, efforts were made to transfer a restorer from B. juncea (Zem) into B. napus. Five lines with uniform male fertility in the pol cytoplasm were obtained after four generations of backcrossing and three generations of selfing. The chromosome numbers of 35 plants chosen from the five fertile lines were determined at meiosis.

The chromosome numbers of these 35 fertile plants with the pol cytoplasm ranged from 37 to 40 (Table 6.3). The majority of these plants (60%) had 38 chromosomes forming 19 bivalents at diakinesis. The rest (40%) were aneuploids with 37, 39, and 40 chromosomes, respectively. These aneuploids were not morphologically distinguishable from normal plants (2n=38). The percentage of aneuploids which remained after several generations of backcrossing and self-pollination was unexpectedly high. The number of plants (14%) with 39 chromosomes suggests that selection for a chromosome from B. juncea which carried a restorer gene for the pol cytoplasm occurred in the backcross and self-pollination generations. These plants (19 II + I) would produce

progenies with 40, 39, and 38 chromosomes and irregular meiosis could produce progeny with 37 chromosomes (Khush 1973).

Any disomic progeny (2n=38) without the gene from B. juncea should be male sterile and would be eliminated by selection. Therefore, substitution of a pair of chromosomes or translocated portions of chromosomes from B. juncea should have occurred in the male fertile plants with 38 chromosomes, and the fertile plants with 40 chromosomes would possess an additional pair of chromosomes from the same species. Further research is needed to confirm these indications.

A total of 80 F_1 plants from crosses involving sterile plants with the pol cytoplasm and plants from four of these five fertile lines produced only undeveloped anthers and were sterile. However, the 20 F_1 offsprings involving the pollen parent from the other fertile line (line 7-10 in Table 6.3) were fully fertile. These results were unexpected and could not be interpreted properly on the basis of data which was available. However, the chromosome number of the plant which restored the fertility was 40 and that for its fertile progeny was 39. Thus, the fertility restoration for the pol cytoplasm may be associated with a gene or genes located on an additional chromosome derived from B. juncea. (This paper will be submitted to Can. J. Plant Sci.)

TABLE 6.3

Chromosome numbers in plants of the five fertile lines derived from the cross between B. juncea and B. napus.

Line Number	Total Plants	Chromosome number 37	38	39	40
7-1	5	-	5	-	-
7-2	4	2	1	1	-
7-3	4	-	4	-	-
7-6	18	4	10	3	1
7-10	4	-	1	1	2
Total	35	6	21	5	3
Percent		17	60	14	9

A CYTOGENETIC STUDY OF MONOSOMIC PLANTS IN RAPE (BRASSICA NAPUS L.)

Abstract

Two types of monosomic plants were discovered among the backcrossed progenies from interspecific crosses between Diplotaxis muralis and Brassica napus, and between B. juncea and B. napus. They were designated as mono-1 and mono-2, respectively. Morphologically they were indistinguishable from their sib disomic plants. Seed production of both mono-1 and mono-2 plants was normal. Cytological examination revealed that most pollen mother cells (average 85%) of mono-1 plants formed 18 bivalents plus a univalent at diakinesis, while the rest (average 15%) formed 17 bivalents plus a trivalent. The univalent was submetacentric and its two arms were always stained lighter than the centromeric region. Later meiotic stages in mono-1 plants appeared normal. The plants of mono-1 produced two types of pollen grains which were different in size. Both large and small pollen grains stained dark with a I_2-KI solution. Meiotic behavior of mono-2 plants was similar to that of mono-1 plants, but the frequency of trivalent formation was higher (average 62%). The univalent was longer than the two chromosomes it paired with to form a trivalent. Pollen produced on mono-2 plants was uniform in size and comparable to that of the normal disomics.

Introduction

Monosomics have been widely used for genetic and breeding research in plants (Riley and Law 1984). They are particularly useful in the mapping of genes (Sears 1953), in the determination of linkage groups to their respective chromosomes (Sears 1966), in the studies of genetic control of chromosome pairing (Riley and Chapman 1958), and in the manipulation of chromosomes (Sears 1953, Unrau et al. 1956, Kuspira 1966).

Monosomics can occur spontaneously in normal disomics (Riley and Kimber 1961, McGinnis 1962). They can also originate from aneuploids, intervarietal and interspecific crosses, and translocation heterozygotes (Khush 1973).

The occurrence of monosomic plants has never been reported previously in rape (Brassica napus). In our study of cytoplasmic male sterility in the rape, interspecific crosses were made between Diplotaxis muralis and Brassica napus, and between B. juncea and B. napus. The F_1 hybrids of these crosses were backcrossed to B. napus for several generations. A few monosomic plants were discovered in the backcross progenies. This paper reports the cytogenetic study of these monosomic plants.

Materials and Methods

The seed stock of D. muralis was obtained from Japan. In order to utilize the cytoplasm from D. muralis to produce a cytoplasmic male sterile line (Hinata and Konno 1979), a cross was made between D. muralis and B. napus cultivar Regent at the Department of Plant Science,

University of Manitoba. These hybrids were further backcrossed to Regent for six generations. Sterile plants from the last backcross were then crossed to a cultivar Kosa. Upon examination of meiosis in 23 fertile plants, one plant showed a chromosome pairing of 18 bivalents plus one univalent. The status of monosomy was further confirmed by mitotic chromosome counts made in root tip. This plant was self-pollinated. Eight monosomic plants were found among 42 selfed offsprings.

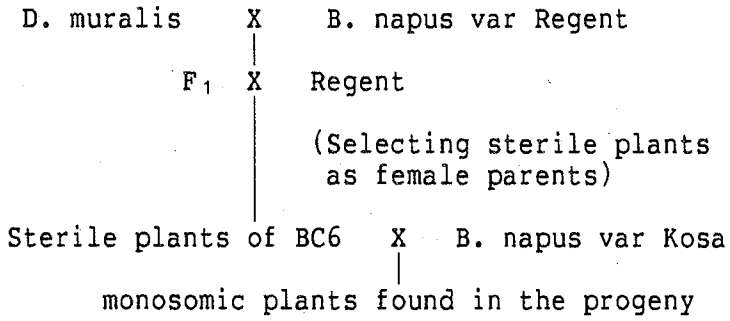
In order to transfer fertility restorer gene(s) from B. juncea, a cross was made between the Polima cytoplasmic male sterile plants of B. napus and B. juncea cultivar Zem. Backcrosses were made in the manner outlined in Fig. 7.1. The fertile offsprings from the last backcross generation were selfed for three generations to select for stabilized restorer lines. Among 35 plants from the last selfed generation, six plants were found te be monosomic, 21 disomic, 5 trisomic and three tetrasomic.

For convenience of discussion, monosomics derived from crosses between D. muralis and B. napus, and between B. juncea and B. napus will be referred to as mono-1 and mono-2, respectively.

All the plants used in this study were grown in the greenhouse. For cytological investigation, flower buds were fixed in a "Carnoy" solution consisting six parts of absolute alcohol, three parts of chloroform, and one part of glacial acetic acid (v:v:v). Anthers from the fixed buds were smeared in a drop of aceto-carmine. A minimum of 50 pollen mother cells (PMC) from each monosomic plants was observed with a Zeiss

Photomicroscope II under phase contrast illumination. Photographs were taken from temporary slides using Kodak Technical Pan 2415 film.

A. Origin of monosomic plants from the cross between D. muralis and B. napus.



B. Origin of monosomic plants from the cross between B. juncea and B. napus.

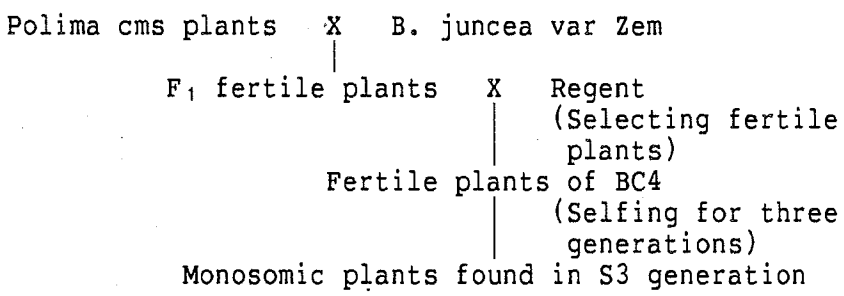


Figure 7.1: Interspecific crosses from which monosomic rape plants originated

Results

Both mono-1 and mono-2 plants were morphologically indistinguishable from regular disomic plants of B. napus. Fertility of these plants, as judged by pollen stainability in a I_2-KI solution and seed set, was also normal. The monosomic plants were identified primarily by meiotic analysis. Mitotic chromosome counts were made in only a few plants for further verification of their monosomic identity.

The meiotic behavior of the monosomic plants was examined in detail at diakinesis. At this stage, chromosomes were well spread and pairing configurations could easily be identified. Data of chromosome pairing is presented in Table 7.1. Most microsporocytes of the mono-1 plants (average 85%) formed 18 bivalents and a univalent (Fig. 7.2). The univalent was submetacentric and always stained darkly in the centromeric region, but both arms stained lightly. The rest of the microsporocytes (average 15%) formed one trivalent in addition to 17 bivalents (Fig. 7.3). The trivalent usually assumed the shape of a chain. However, among the individual mono-1 plants, the trivalent frequency varied widely from 38% in the original plant to 2% in its two offsprings, plants 3 and 7 (Table 7.1).

At metaphase I, chromosomes in the majority of the PMC's aligned regularly at the equator to form a metaphase plate. It was also observed that in some microsporocytes, the univalent laid outside of the metaphase plate or oriented towards one of two poles. As the chromosomes were highly condensed at this stage, reliable identification of the trivalent could not be made. At anaphase I, a chromatin bridge

TABLE 7.1

Chromosome pairing in mono-1 and mono-2 plants discovered in the backcrossed progeny from the crosses between D. muralis and B. napus and between B. juncea and B. napus

		Chromosome configuration			
plant number	No.cells	18II + 1I	Percent	17II + 1III	Percent
(Mono-1) 1	57	35	61	22	39
2*	69	66	96	3	4
3	57	56	98	1	2
4	61	51	84	10	16
5	80	71	89	9	11
6	46	31	67	15	33
7	60	59	98	1	2
8	77	63	82	14	18
9	80	69	86	11	14
Total	565	501	85	64	15
(Mono-2) 1	72	31	43	41	57
2	64	19	23	65	77
3	69	41	59	28	41
4	62	22	35	40	65
5	37	9	24	28	76
6	85	34	40	51	60
Total	409	156	38	253	62

* Plant number 2 to 9 are the selfed progeny of plant number 1.

was observed in a few microsporocytes. However, it is difficult to determine if the formation of the bridge was related to misdivision of the univalent. At late anaphase I, all cells examined contained 18 chromosomes in one pole and 19 in the other (Fig. 7.4). The second meiotic division appeared normal. At anaphase II, two nuclei had 18 chromosomes and the other two had 19 chromosomes.

Examination of mature anthers of the mono-1 plants revealed that there were two types of pollen grains (Fig. 7.7) which differed in size and occurred approximately in a 1 : 1 ratio (253 large : 279 small). Both large and small pollen grains were stained darkly with the I_2-KI solution, indicating that starch was accumulated in all the pollen. The large pollen grains were comparable to that of disomic plants.

Microsporocytes of the mono-2 plants showed similar meiotic behavior, 18 bivalents plus a univalent or 17 bivalents plus a trivalent (Table 7.1, Fig. 7.5, 7.6). The univalent was different from that found in the mono-1 plants in that its two arms were always deeply stained. Although this univalent frequently formed a trivalent with another pair of chromosomes, it did not have any morphological similarity to its pairing partners (Fig. 7.6). The occurrence of trivalents was high (average 62%). The subsequent stages of meiosis in the mono-2 plants resembled those in the mono-1 plants. Mature pollen of the mono-2 plants was uniform in size and similar in dimension to that of disomic plants.

Figure 7.2: Diakinesis of mono-1, showing 18 bivalents plus a univalent (indicated by an arrow). X 1200.

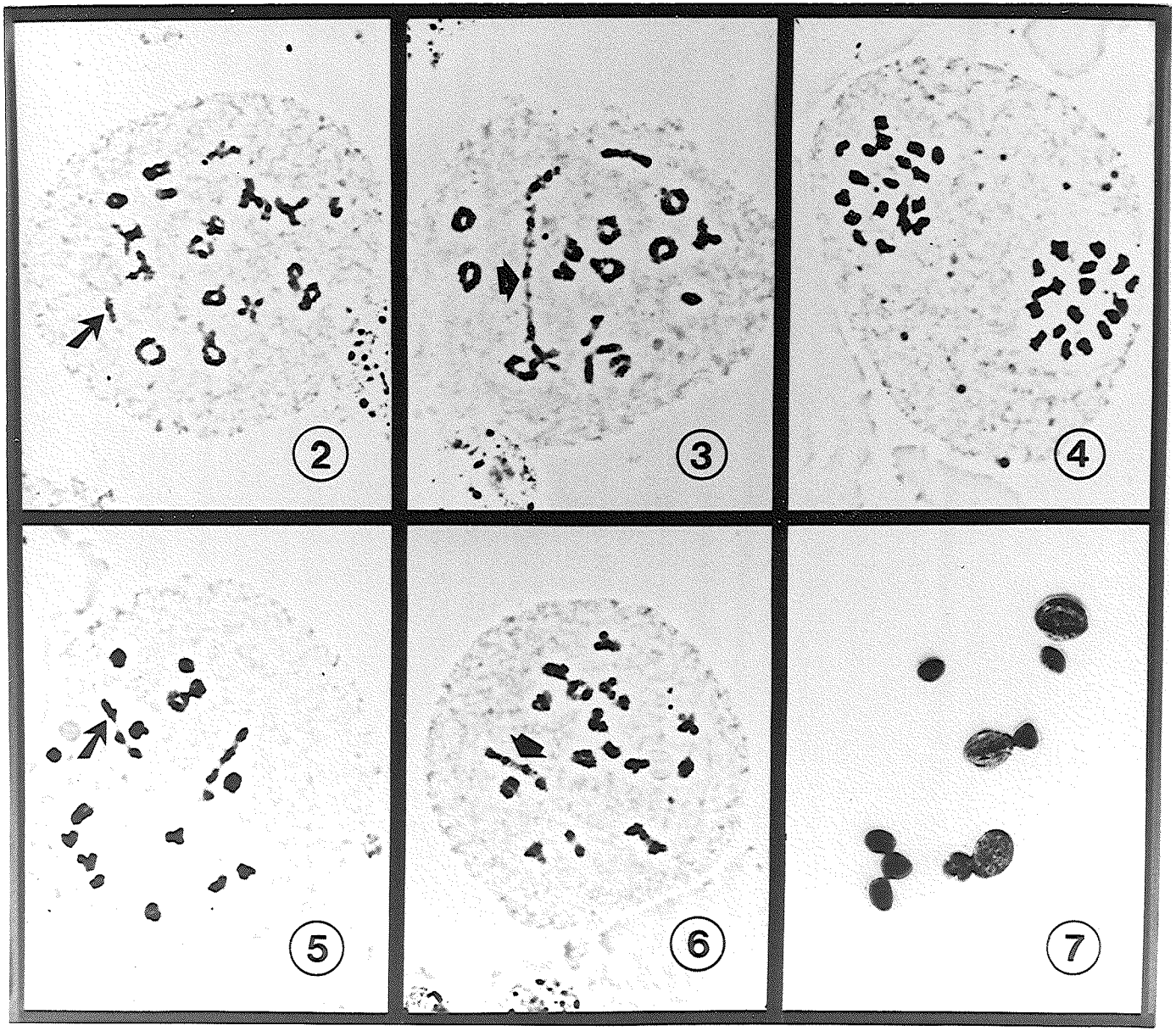
Figure 7.3: Diakinesis of mono-1, showing 17 bivalents plus a univalent (indicated by an arrow). X 1200.

Figure 7.4: Anaphase I of mono-1, showing 19 chromosomes at one pole and 18 chromosomes at the other. X 1200.

Figure 7.5: Diakinesis of mono-2, showing 18 bivalents plus a univalent (indicated by an arrow). X 1200.

Figure 7.6: diakinesis of mono-2. showing 17 bivalents plus a trivalent (indicated by an arrow). X 1200.

Figure 7.7: Large and small pollen grains produced on mono-1. X 350.


2
3
4
5
6
7

Discussion

Monosomics are commonly found in polyploid species owing to the fact that the loss of a chromosome can be compensated by the presence of its homoeologous partner. Complete sets of monosomics have been reported in wheat, oats, and tobacco (Khush 1973). B. napus is an amphidiploid species consisting of the A and C genomes derived from B. campestris and B. oleracea, respectively (U 1935). Available evidence indicates that the A and C genomes have a high degree of homology (Prakash and Hinata 1980). Therefore, high tolerance to monosomy in B. napus is expected. This view is substantiated by the fact that both mono-1 and mono-2 plants were not different morphologically from their disomic sibs. Furthermore, seed set of both mono-1 and mono-2 was normal. This evidence suggests that it is possible to obtain a whole set of monosomics in B. napus.

In our breeding program, consistent selection pressure for male sterility was applied at every backcross generation from the cross between D. muralis and B. napus. Examination of sterile plants revealed that they were all trisomic, which led to the conclusion that the male sterility was associated with this extra chromosome (Fan et al. in press). Similarly, trisomics were also found in the last selfed generation from the cross between B. juncea and B. napus as a result of selection for male fertility. It was considered that the fertility restoring gene for the pol cms was located on this extra chromosome from B. juncea. Trisomics have been known to be a source of monosomics due to occasional failure of homologous pairing, non-disjunction of trivalent, or loss of univalents during meiosis (Sears 1954, Brown and Endrizzi 1964, Khush 1973).

The morphological similarity between monosomics and disomics makes it difficult to identify monosomics in a large population of plants. For mono-1, pollen size can be used as a criterion, while mono-2 plants can only be detected by cytological examination. Although the pollen produced on mono-1 plants was different in size, they were all stained darkly with a I_2-KI solution. This indicates that they were all viable. Since the large sized pollen closely resembled that of the disomics, they are considered to carry a normal chromosome complement of 19 (n) chromosomes. Accordingly, the small pollen would have 18 (n-1) chromosomes.

Nullisomics were expected to occur in the selfed progeny of both mono-1 and mono-2. However, they have not been observed. Probably the number of plants examined was too small. The other possibility is that certation of pollen grains or selection against pollen with a (n-1) chromosome number might have acted to prevent the transmission of (n-1) male gametocytes. If this is true, the occurrence of monosomics in the selfed progeny would be attributed solely to the transmission of (n-1) gametes from the female side.

Mono-1 and mono-2 are different monosomics as they differ in origin, chromosome morphology, chromosome pairing behavior, as well as pollen type. In mono-2, the univalent was found to be longer in length than the bivalent it frequently associated with to form a trivalent. If high frequency of trivalent formation is an indication of homology between chromosomes of the univalent and the bivalent, morphological difference was probably caused by structural modification. However, no valid conclusion can be drawn without further cytological and genetic study. (This paper has been submitted to Can. J. Genet. Cytol..)

GENERAL DISCUSSION AND CONCLUSIONS

The discovery of cytoplasmic male sterility (cms) and finding of substantial heterosis for seed yield in F_1 hybrids have stimulated the interest in development of hybrid cultivars in rape (B. napus). Four cms systems conditioned by the mur, ogu, nap, and pol cytoplasms have been reported. The characters of the four cms systems were investigated using cytological and histological techniques. The inheritance of restoration for fertility and stability of male sterility were also studies. Information obtained is useful not only in evaluation of utility of these systems in a hybrid breeding program but also in classification and characterization of these systems.

The establishment of a male sterility system in B. campestris using the cytoplasm from D. muralis (Hinata and Konno 1979) suggests the possibility of using this cytoplasm to produce cms lines in B. napus. The present study reveals that the male sterility observed in the backcrossed generations involving the cross between D. muralis and B. napus is associated with an extra chromosome present in the male sterile plants, thus indicating that this male sterility is not cytoplasmically conditioned, or more likely B. napus has restorers in the C genome. Therefore, the potential of using the mur male sterility system in a hybrid breeding program is very limited.

Previous reports (Heyn 1979, Rousselle 1979) and results from this study indicate that all rape cultivars tested are maintainers for the

ogu cms system. The absence of fertility restorers for the ogu cms in B. napus, is the major obstacle in utilization of this cms system to breed hybrid cultivars. Since fertility restorer genes for the ogu cms system are indigenous to radish (Bonnet 1975), transfer of these genes from R. sativus to B. napus is essential if the ogu cms system is to be used for seed production in F_1 hybrids.

To characterize and classify the pol cms system, its various attributes were investigated and were compared with the ogu and nap cms systems. Histological investigation, the stability of male sterility, and the pattern of fertility restoration indicate that the male sterility conditioned by the pol cytoplasm is different from that conditioned by the ogu and nap cytoplasms. Thus, the pol and nap cytoplasms are two different types of male sterility inducing cytoplasm in B. napus.

Since maintainer and restorer lines are available for both the pol and nap cms systems, they can be used to produce F_1 hybrid seeds. The major drawback of the two systems is the inconsistent expression of male sterility under influence of temperature. However, variation in response of male sterile plants to high temperatures suggests that selection of genotypes with a higher level of male sterility is possible. Before the pol and nap cms systems can be used efficiently for commercial hybrid seed production, further research in needed on the performance of male sterile plants in the field, on the incorporation of quality factors (low erucic acid and low glucosinolate) into the male sterile, maintainer, and restorer lines, and on the level of cross-pollination between male sterile and restorer plants.

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