CYTOPLASMIC MALE STERILITY IN TURNIP-RAPE (*BRASSICA CAMPESTRIS* L.)

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by

Matti Sovero

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ABSTRACT

Matti Sovero, Ph.D., The University of Manitoba, January 1987. Cytoplasmic male sterility in turnip-rape (Brassica campestris L.). Major Professor, B. R. Stefansson.

Forty one strains and cultivars of Brassica campestris L. were tested for their ability to either maintain complete male sterility or restore fertility in crosses with male sterile lines of B. napus with cytoplasms mur, nap, ogu or pol. Progenies were grown at two locations, Winnipeg, Canada and Jokioinen, Finland.

All progenies with ogu cytoplasm were male sterile, showing abnormal anther development and no pollen shed. Thus no fertility restoration for ogu cytoplasm was present in B. campestris tested.

Progenies with mur cytoplasm had normal anthers with abundant pollen shed but a few male sterile plants were observed. The sterility may be associated with a supernumerary chromosome.

None of the F_1 progenies from crosses involving nap cytoplasm were completely male sterile. The progenies grown at both locations expressed better anther development in Winnipeg than in Jokioinen, which indicates that there is an interaction between location and the expression of male sterility in nap cytoplasm. Both male sterile and male fertile progenies were observed in the F_1 generation of crosses

involving pol cytoplasm. The B. campestris strains of Chinese origin produced predominantly sterile F_1 progenies and could be classified as maintainers. The cultivars of Swedish origin produced a high frequency of progenies with either normal or almost normal anther development and abundant pollen shed, which indicates a high frequency restorer of genes.

Reciprocal crosses were made among four cultivars of B. campestris with cam cytoplasm, and heterozygous male fertile individuals of B. campestris (pol). Three individuals of each cultivar were used as parents for crosses. Varying frequencies of male sterile plants were observed in the progenies with pol cytoplasm, while all plants in the progenies with cam cytoplasm were male fertile. The results support the assumption that the male sterility associated with pol cytoplasm is controlled by a cytoplasmic-genic system. Sterility was associated with small petals and slightly irregular growth of the pistils in some cases. Sterile plants were also observed to be taller than the fertiles.

Selection for the maintenance of male sterility in crosses with B. campestris (pol) was practiced using cultivars Nopsa, R-500 and TL-15 of the same species. Significant response to selection was observed in all three cultivars, which indicates that heritable variation in the ability to maintain complete male sterility in pol cytoplasm exists in these cultivars.

Twenty strains and cultivars of oilseed B. campestris from five countries were studied for their ability to restore fertility in

crosses with male sterile B. campestris (pol). The highest frequencies of male fertile plants were found in the progenies of the Swedish cultivars Ante and Sv 03201. The Chinese cultivar 'Golden', as well as Indian cultivars 'BSH-1' and 'Pusa Kalyani', did not produce any male fertile progeny. These results confirm the evidence from the interspecific crosses between B. napus and B. campestris that restoration of male fertility for pol cytoplasm is available in B. campestris.

The inheritance of the restoration of full fertility was studied in progenies of test crosses between male fertile and male sterile B. campestris (pol) and the selfed progeny from these crosses. At least two genes were found to be able to restore fertility independently, and at least one of them was completely dominant to the maintainer. A minimum of two recessive alleles are needed to maintain complete male sterility in B. campestris with the pol cytoplasm. The genetic background may have a significant effect in modifying the level of fertility or sterility caused by the major genes.

The effect of temperature and senescence on the male sterility of B. campestris (pol) was investigated. The day/night temperature regimes used were 14/6°, 22/14°, and 30/22°C. The sterility of the anthers was observed three times: at the beginning of flowering, two days later, and at the end of flowering on the main raceme. A decrease in the number of male sterile plants was observed in low temperatures and toward the end of the flowering, but complete reversion to male fertility did not occur.

The results indicate that the male sterility associated with the pol cytoplasm could be used for breeding hybrid cultivars of B. campestris. Both maintainers of stable male sterility and restorers of fertility are present in the species.

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I CHAPTER

INTRODUCTION

Turnip-rape (Brassica campestris L.) is an important oilseed crop in India, China, Northern Europe and Western Canada. Summer forms of this crop are grown in Canada, Sweden and Finland in areas where the short growing season requires a crop that matures before the fall frosts.

Winter turnip-rape, once common in northern Europe, is now grown only on a small area in Sweden. It has mainly been replaced by summer rape (B. napus L.) and summer turnip-rape in areas where overwintering used to be a problem. Elsewhere the higher yielding winter rape has supplanted winter turnip-rape.

The different forms of B. campestris grown in India comprise three main types: yellow sarson, brown sarson, and toria. Toria, mostly grown on the foothills of the Himalayas is an early maturing crop, usually sown in September and harvested by the middle of December. The sarson types are cultivated as winter crops. They are sown in October or early November and harvested in March or April (Prakash 1980).

The introduction of cultivars with a low erucic acid content and later the development of "canola" type cultivars with low erucic acid

and glucosinolate contents has greatly stimulated the demand for rapeseed products, the result has been an increase of the acreage of both rape and turnip-rape, especially in Canada and northern Europe. Although much work is still being done to improve further the quality of the crop (Stefansson 1983), these changes have induced plant breeders to pay increasing attention to the yielding ability as well as other agronomical characters of the crop.

The use of conventional breeding methods has gradually increased the yields of predominantly cross-pollinating turnip-rape. Since significant heterosis for seed yield has been observed in intervarietal hybrids (Patnaik and Murty 1978, Hutcheson et al. 1981), it is likely that faster progress toward increased yields will be achieved by introducing hybrid cultivars.

The efficient production of hybrid cultivars requires a reliable mechanism for pollination control. Incompatibility systems are widely used for hybrid production in Brassica root and vegetable crops (Ryder 1979, Hinata and Nishio 1980). Since the maintenance of homozygous lines is both difficult and costly, the system does not appear feasible for hybrid production in oilseed turnip-rape.

Cytoplasmic male sterility (CMS) has been used in many crops and probably offers the best prospects for use in developing hybrid cultivars of turnip-rape as well. Several CMS systems have been reported in the genus Brassica, including the ogu, nap, mur and pol systems. The ogu cytoplasm was first observed in an unidentified Japanese strain of radish (Raphanus sativus L.) and the genomes of several Brassica species have since been introduced into the ogu

cytoplasm by means of repeated backcrosses (Bannerot et al. 1974, Williams and Heyn 1981). The nap CMS involving the male sterility inducing cytoplasm of B. napus was found independently in England and Japan in F_2 generations from intervarietal crosses (Shiga and Baba 1971, Thompson 1972). Hinata and Konno (1979) observed that the mur cytoplasm of the sand rocket (Diplotaxis muralis (L.) DC.) causes male sterility in B. campestris. The male sterility inducing effect of pol cytoplasm on B. napus was first discovered by Fu (1981). This cytoplasm was originally obtained from the Polish rape cultivar 'Polima' and the genome of B. campestris was introduced into the cytoplasm as part of the present project.

The focus of this project was on the problems encountered in a hybrid breeding program. Most of the research was conducted in combination with a B. campestris breeding project. The main objective of the study was 1. to compare pol cytoplasm with mur, nap and ogu cytoplasms with respect to their use in breeding hybrid turnip-rape; 2. to examine the fertility restoration and sterility maintenance systems for pol cytoplasm in B. campestris; 3. to study the inheritance of male sterility and fertility restoration in pol cytoplasm; and 4., to determine the effect of temperature on the expression of sterility. The information obtained was used to evaluate the potential of pol cytoplasm for developing hybrid cultivars of B. campestris.

II CHAPTER

LITERATURE REVIEW

2.1 Taxonomy of Brassica

The genus Brassica includes six presently cultivated species. Based on their chromosome numbers these species can be divided into two groups, the diploids and the polyploids (Prakash and Hinata 1980).

The diploid species are Brassica nigra (L.) W.D.J. Koch (n = 8), B. oleracea L. (n = 9) and B. campestris L. (n = 10). These are regarded as aneuploid forms of a common ancestral species (Manton 1932). There is much evidence to support the idea that this species is itself a polyploid, and that the basic chromosome number of the entire genus was originally six (Alam 1936, Thompson 1956, Röbbelen 1960, Prakash 1973). According to this theory, these so-called diploid species are, in fact, secondarily balanced polyploids.

The relationships between the diploid and polyploid species were discussed by Morinaga (1931, 1933, 1934a, 1934b) and by U (1935). Morinaga presented a hypothesis showing how the three polyploid species resulted from pairwise combinations of the three diploids. U (1935) demonstrated the validity of this hypothesis by successfully synthesizing B. napus L. by crossing B. campestris and B. oleracea.

Subsequently B. juncea (L.) Czern. has been reconstituted (Frandsen 1943, Ramanujam and Shrinivachar 1943) as well as B. carinata A. Br. (Frandsen 1947). U (1935) presented the relationships between the six cultivated species of Brassica in the form of a triangle, generally known as the triangle of U (Figure 2.1).

The definition of the relationships within Brassica, and also among other closely related crucifers, has provided a subject of much interest for geneticists and botanists, as well as for plant breeders. Homology has been found to exist among chromosomes of the genera Brassica, Diplotaxis, Eruca, Raphanus and Sinapis. In some cases, a greater degree of homology prevails between genera than among the interspecific crosses within a genus (Mizushima 1980). Restriction enzyme analyses of chloroplast DNA seem to indicate that boundaries between Brassica, Raphanus and Sinapis are unclear (Palmer et al. 1983).

Research into the taxonomy of the cultivated species of Brassica can greatly further our understanding of the evolution of plant species and associated cytological phenomena. These research findings also directly benefit the plant breeder. In many cases, the breeder is able to widen considerably the variation at his disposal by synthesizing allotetraploids from their ancestral species (Namai et al. 1980, Olsson and Ellerström 1980, Prakash 1980). At least one new crop, hakuran, a heading type of swede (B. napus) developed in Japan, has been created in this way. It is an allotetraploid hybrid between Chinese cabbage (B. campestris ssp. pekinensis) and common cabbage (B. oleracea) (Nishi 1980). Various types of Brassicoraphanus,

allopolyploids derived from crosses between Brassica species and Raphanus sativus, are given as examples of potential crop plants (McNaughton and Ross 1978, Olsson and Ellerström 1980).

Another interesting feature found in the genus Brassica is so-called parallel variation (Tsunoda and Nishi 1968). Similar types of diversity exist in cultivars of B. juncea, B. oleracea and B. campestris. For example, for most of the types of common cabbage (B. oleracea) grown in Europe, a parallel counterpart can be found from among the cultivars of B. juncea and B. campestris used in East Asia (Nishi 1980). Of course the existence of such widespread and unidirectional variation does nothing to lighten the load of taxonomic confusion. For example, phenotypes which in the case of B. oleracea are considered as variants, are in the case of B. campestris sometimes designated as subspecies, or even distinct species, while in B. juncea they have been left entirely devoid of taxonomic status (Prakash and Hinata 1980). Nevertheless, such variation offers the breeder a wide scope of interesting possibilities.

2.2 Heterosis in Brassica Oilseed Crops

2.2.1 Heterosis and Hybrid Breeding

In crops grown for their seed yield, the main motivation for using hybrid cultivars is usually the associated increase in seed yield. The occurrence of heterosis for yield within the species is a prerequisite for embarking on a hybrid breeding programme. Heterosis can be evaluated in several ways (Boland and Walcott 1985). True

heterosis is expressed as a percent of the mid-parent value. For breeding purposes, the yield is better expressed as percent of the higher yielding parent or high-yielding standard cultivar. For commercial hybrid cultivars the comparison with a standard cultivar is the most meaningful test. A review of studies on heterosis in the most important oil crops in the genus Brassica follows.

2.2.2 Heterosis in Oilseed Rape

The existence of significant heterosis in crosses between cultivars of rape (B. napus) has been reported by several researchers. Heterosis or hybrid vigor is the increase or decrease in the expression of a trait in F_1 in comparison to the mid-parent value. Alternatively, either parent or another standard cultivar can be used for comparison. In his studies of crosses between Swedish and Japanese cultivars, Olsson (1954) observed heterosis with respect to plant height. Heterosis for seed yield was also discernible, though it was not statistically significant.

Schuster (1969) found that hybrids of selfed winter rape lines produced yields on average 30 % higher than for the parents. The differences between different hybrids were significant. In investigations on inbreeding depression and heterosis in winter rape, Schuster and Michael (1976) reported significantly higher yields in hybrids over the standard varieties.

Shiga (1976) studied 62 hybrids between European and Japanese winter rape cultivars and found heterosis with respect to seed yield, seed size, length of flowering and number of side branches.

Based on information available at that time, Röbbelen (1980) recommended that the development of hybrid rape cultivars should proceed as quickly as possible.

Lefort-Buson and Dattée (1982) tested 140 winter rape F_1 hybrids and found that some of them produced 50 % more seed than their parents. On average, hybrids outyielded their parents by 23.5 %. Lefort-Buson (1982) also found significant heterosis in spring rape. Five cultivars were crossed in 5 x 5 diallel design. In a field trial with four replications the hybrids produced on average 302 kg/ha in excess of the more productive parents. The average yield of the hybrids was 2885 kg/ha. The cultivar 'Abumasari', of Asiatic origin, was found to have the highest general combining ability.

Sernyk and Stefansson (1983) crossed Regent, then the most widely grown cultivar in Canada, with various foreign cultivars. The hybrids were compared with their parents in field trials of three replications for two years. The best combinations, Gulliver x Regent and Karat x Regent, outyielded the higher yielding parent, Regent, by 41.5 % and 43 %, respectively. The highest yield harvested was 3072 kg/ha (Gulliver x Regent). In both of the higher yielding combinations one of the parents was of Swedish origin. Grant and Beversdorf (1985) conducted a 6 x 6 diallel cross at two locations in Ontario. Heterosis for seed yield of up to 72 % over the superior parent was observed in the F_1 hybrids. The conclusion from both of these studies was that reasonable potential for producing high yielding hybrid cultivars in oil seed rape exists, but the possibilities of utilizing the heterosis commercially will depend on the successful development of efficient mechanisms for pollination control.

2.2.3 Heterosis in Brown Mustard

Brown mustard (*B. juncea*) is an important oil crop in India where much attention is devoted to crop improvement (Prakash 1980). In fact most of the studies dealing with heterosis in brown mustard have been carried out in India.

Singh (1973) used six commercial varieties and four breeding strains as parents in diallel crosses. All 90 F_1 progenies and their parents were grown in four replications for two years. In six crosses the hybrid significantly outyielded the better parent, on average by 49 %. Heterosis was also detectable with respect to branching.

Asthana and Singh (1973) produced two hybrids in crossing blocks using as the female parent a mutant line in which the pistil protrudes from the buds. Both hybrids produced significantly higher yields than the pollen parent or high yielding commercial cultivar Laha 101, used as a standard. In this case also the hybrids were found to branch more freely than their parents.

Gupta (1976) compared four hybrids with their parents in replicated field trials. In all cases the heterosis with respect to yield was positive, although in only one case was the advantage over the better parent significant (62.8 %). Positive heterosis was observed for branching and the number of pods, but not for seed size.

Banga and Labana (1984) produced 139 hybrids using 43 Indian and 15 European cultivars of brown mustard as parents. In two years of trials the hybrids yielded on average 18.8 % more than the superior parent. The greatest difference between the hybrid and the higher yielding parent was 262.7 %. The number of pods on the main raceme

and the number of secondary branches was significantly higher for the hybrids than for the parents.

A common feature of all the studies in B. juncea is the fact that the trials were established at a fairly low planting density, and that the high yield of the hybrids is largely attributable to their abundant branching. Prakash (1980) found this aggressive habit of growth to confer an advantage, especially on plants subject to drought stress. Guan (1980) and Sernyk and Stefansson (1983) suggest on the basis of their observations, that also hybrids of B. napus utilize soil water resources more efficiently than the parental lines.

2.2.4 Heterosis in Turnip-rape

Devarathinam et al. (1976) studied 150 F_1 hybrid progenies whose parents represented the three main types of Indian turnip-rape (B. campestris) cultivars: brown sarson, yellow sarson and toria. Allthough no yield measurements were taken, pronounced heterosis was observed for various components of yield, especially in crosses between cultivars of different types. Most of the heterosis was expressed for the number of secondary branches.

Patnaik and Murty (1978) made crosses among outbreeding and inbreeding cultivars of the brown sarson type. The inheritance of all the observed characters displayed both additive and non-additive gene effects. Heterosis was either absent or marginal for most of the characteristics. However, variable amounts of heterosis for yield were observed, the maximum being 42.5 %.

Hutcheson et al. (1981) examined spontaneous crosses between the high erucic, inbreeding cultivar R-500 selected from an Indian population of yellow sarson, and the low erucic, outcrossing cultivar Candle. The cross progenies were readily identifiable on account of their intermediate content of erucic acid. The hybrid outyielded the better parent, in this case Candle, by 46 %. The protein content of the hybrid was 4.1 % higher than that of Candle.

In these examples of B. campestris significant heterosis was observed when the parents of the F_1 hybrids were of widely differing types, and in most cases when at least one of the parents was of a self-pollinating type. It is likely that in out-crossing turnip-rape there is so much genetic variation within the cultivars that little or no heterosis is expressed in progenies from crosses between cultivars of the same type and geographical origin.

Singh and Gupta (1985) proposed a statistical method for screening turnip-rape breeding material for crosses likely to exhibit various types of heterotic progeny. The method is based on the estimation of genetic-environmental interaction for the potential parents, which can then be classified into groups. By the application of such a system, it might be possible to use simple means to select strains for a hybrid program with an optimal combination of genetic diversity, yielding potential and adaptation to various growing conditions and methods of cultivation.

2.3 Pollination Control Systems of of Cruciferous Oil Crops

2.3.1 Techniques to control Pollination in Hybrid Production

In the production of hybrid seed, a satisfactory degree of crossing between the chosen parents can be ensured in various ways. There are six basically different techniques: natural dioecity, manual or mechanical emasculation of the female parents, chemical emasculation, genic male sterility, cytoplasmic-genic male sterility (abbreviated to CMS) and self-incompatibility.

The Brassica oil crops are naturally monoecious, and manual emasculation is not economically feasible in rapeseed. Thus these two methods cannot be used in commercial hybrid production.

Van der Meer and van Dam (1979) attained promising results by the use of gibberellic acid for emasculating various types of cabbage. The degree of success, however, varied according to the type of crop and even the cultivar, so that this technique may be applicable only in special cases.

Genic male sterility occurs widely within the genus Brassica (Shiga 1980). Takagi (1970) investigated its potential for the production of F_1 seed. The main drawback to the practical application of male sterility is that it can only be maintained in a segregating population, which means that male fertile individuals must always be rogued from the population to be used as the seed parent in the crossing. This makes the procedure tedious and expensive. In systems based on balanced tertiary trisomy, such as those developed for barley (Ramage 1983), there is no such drawback, but it appears that such systems have not yet been developed for crucifers.

Cytoplasmic-genic male sterility, which in accordance with general convention will be termed cytoplasmic male sterility, differs from strictly genic male sterility in that the sterility gene or genes are expressed only in certain types of cytoplasm. A sterile line can thus be maintained by pollinating it with a line having the same nuclear genotype but in different cytoplasm. In order to make hybrid production feasible for crops grown for their seed yield, a dominant restorer gene is also needed, i.e. a gene in whose presence plants with sterile cytoplasm will produce pollen normally, or almost normally. The following section will deal with the cytoplasmic male sterility systems to be found in various species of Brassica.

A sporophytic incompatibility system is widely used for hybrid production in Brassica root and vegetable crops (Thompson 1978, Ryder 1979, Hinata and Nishio 1980, Pearson 1983). It has been presented in a useable form for hybrid seed production in rape (Thompson et al. 1983). One advantage of the sporophytic system over systems based on male sterility is that hybrid seed can be harvested from both of the parent lines. The application of the system, however, is not without its problems.

The most serious difficulty is that of producing and maintaining the self-incompatible lines. Since homozygotic lines are not normally capable of intrapollination, special methods must be employed in order for seed to be obtained (Hinata and Nishio 1980). On a small scale, eg. for experimental purposes, it is possible to produce selfed seed by pollinating the flowers shortly before they open naturally, at a time when the self-incompatibility system is not

fully operative. This system of so-called bud pollination was originally developed by Pearson (1929, 1932) and is now used by breeders all over the world. Fu (1981) relates that the method is used in China for the production of selfed rape lines on a practical scale, but mentions in the same context that there, too, efforts are being made to develop less tedious alternatives. The degree of self-pollination can also be increased by raising the carbon dioxide concentration of the atmosphere (Nakanishi and Hinata 1975, Hinata 1975, Taylor 1982). This technique is feasible only in a confined space, eg. a plastic tunnel, another factor which will inevitably raise the costs of seed production. In addition, workers have observed that the carbon dioxide treatment is most effective in the case of lines whose self-incompatibility is inherently weak. Such lines may not be suitable for hybrid seed production (Hinata and Nishio 1980).

The dominance relations in sporophytic incompatibility are often highly complex, and are influenced by many factors, as is the expression of incompatibility in general (Hinata and Nishio 1980). This makes such systems difficult to control and inflexible in practice. According to Hinata and Konno (1979), the existence of incompatibility considerably restricts the use of cytoplasmic male sterility in hybrid breeding, and they proposed as a solution to this problem that only lines capable of self-pollination should be used for this purpose.

Of the Brassica oil crops, turnip-rape (B. campestris) is predominantly outcrossing, while rape (B. napus) and brown mustard

(B. juncea) are self-pollinating (Hinata and Nishio 1980). Some degree of self-incompatibility has been observed in rape as well (Olsson 1960, Thompson et al. 1983). Yellow sarson, a type of turnip-rape grown in some parts of India, is self-pollinating (Prakash 1980). Hinata et al (1983) studied the inheritance of incompatibility in crosses between yellow sarson and outcrossing types of turnip-rape. In addition to S alleles found previously (Bateman 1955, Thompson 1957, Sampson 1957, Richards and Thurling 1973, Sareen and Kakar 1975, Zuberi et al. 1981), Hinata et al. found at another locus a gene they designated M whose recessive allele (m) when occurring as a homozygote permitted self-pollination. The investigators concluded that the S alleles control the identification system associated with the incompatilibity mechanism, while the M genes actually control the germination of the pollen grains on the stigma. It would thus appear feasible to transfer self-incompatibility from yellow sarson to other types of turnip-rape by backcrossing the m allele into them without manipulation of the awkward series of S alleles.

2.3.2 Systems of Cytoplasmic Male Sterility in Cruciferous Crops

2.3.2.1 Ogu Cytoplasm

The first type of cytoplasm observed to cause male sterility in crucifers was found in an unidentified Japanese variety of radish (Raphanus sativus L.) (Ogura 1968). This cytoplasm is now called ogu cytoplasm after its discoverer (Shiga 1980). Typical features of the

sterile radish individuals are small buds, often defective opening of the flowers and curved pistils. Sterility was found to arise through the interaction between the cytoplasm and a recessive gene within the nucleus, and to be manifested primarily in the degeneration of tissues of the tapetum and the resulting arrestation in development of the stamens. No restorer genes were found in Japanese radish cultivars (Ogura 1968). In contrast, a fertility restoring dominant gene is relatively common in European cultivars (Bonnet 1975, 1977). At least in France, ogu cytoplasm has been succesfully used in radish hybrid breeding (Bonnet 1977).

Subsequently, the genomes of cabbage, rape, and various types of B. campestris have been backcrossed into ogu cytoplasm (Bannerot et al. 1974, 1977, Williams and Heyn 1981). In addition to causing male sterility, the foreign cytoplasm was found to result in severe chlorosis of leaves, especially at low temperatures, as well as certain other disturbances (Bannerot et al. 1977). Rouselle (1981) noted that although the leaves are normal in colour at temperatures above 15°C, their chlorophyll content is lower than normal. McCollum (1981) observed that the fertile cytoplasm of radish, designated rap by Shiga (1980) also gives rise to chlorosis in cabbage. These difficulties were overcome by substituting Brassica chloroplasts for Raphanus chloroplasts by means of protoplast fusion, followed by selection for male sterility and absence of chlorosis (Pelletier et al. 1983). These workers also succeeded in combining male sterility with cytoplasmic triazine resistance.

The new type of cytoplasm which has been created in this manner has revealed mitochondria which differ in some respect from the mitochondria of both ogu cytoplasm and of the Brassica species used for the fusion (Chetrit et al. 1985). Examination of these recombinant mitochondria has shown that the factor causing male sterility is most probably situated on the mitochondrial chromosomes, and not in the plasmid-like DNA fragments to be found in the mitochondia, as has also been suggested (Palmer et al. 1983).

The main obstacle to using ogu cytoplasm in breeding cruciferous oil crops is that no fertility restoring genes have been found in any of the Brassica species examined (Bannerot et al. 1977, Williams and Heyn 1981, Fan et al. 1986). So far, no one has succeeded in transferring such genes from the radish to the other species. Heyn (1978) tried to backcross fertility restoring genes from radish into rape, and announced partial success. He claimed that fertility in rape is linked to genes derived from radish which cause a white flower colour. Sernyk (1982) demonstrated that the gene or genes giving rise to white-coloured flowers are situated in a supernumerary chromosome derived from radish, and that the presence of this chromosome did not confer fertility on rape plants with ogu cytoplasm. The "segregation ratios" observed by Heyn were found to depend upon the transference frequency of the supernumerary chromosome during pollination.

2.3.2.2 Mur Cytoplasm

The term mur has been proposed by Shiga (1980) for the cytoplasm of Diplotaxis muralis (L.) DC. This cytoplasm has been observed to cause male sterility in various forms of turnip and Chinese cabbage (Hinata and Konno 1979, Shiga 1980) as well as in rape (Shiga 1980). The sterile plants have flowers with narrow petals and only two nectaries. The opening rhythm of the flowers also deviates from normal. The stamens produce less than half the usual amount of pollen, and do not open sufficiently to allow normal pollination (Hinata and Konno 1979).

Fertility restoring genes could be found in certain cultivars of Chinese cabbage (Hinata and Konno 1979). However, Shiga (1980) was unable to observe any fertility in rape with the mur cytoplasm.

Fan et al. (1985) backcrossed the genome of the Canadian rape cultivar Regent into mur cytoplasm. The backcrossed progenies showed less than 20 % of male sterile individuals. Sterile individuals from the sixth generation of backcrossing were crossed with twelve cultivars of rape. The resulting progenies contained from 0 - 36 % of sterile individuals, but a segregation pattern could not be defined. A cytological examination of the progenies revealed that all the sterile individuals were carrying a single, supernumerary chromosome. The chromosome number of the fertile plants was normal. On the basis of these results it was concluded that the sterility factor in this case was the extra chromosome, most probably originating from D. muralis.

2.3.2.3 Brown Mustard Cytoplasms

Rawat and Anand (1979) found male sterile individuals in a collection of Indian brown mustards (B. juncea). The stamens of the affected plants had either degenerated, or had been converted to petal-like structures. Neither type of stamen produced pollen. The sterility was found to be inherited maternally. In subsequent studies, the same workers were able to find partially effective restoring genes from certain strains of B. campestris, B. napus, B. nigra and B. carinata. It was established that fertility could be improved by selection (Anand and Rawat 1983, Anand et al. 1985a). This type of male sterility is often termed the Anand system, although such nomenclature is neither official, nor does it accord with the system proposed by Shiga (1980). Anand et al. (1985b) have put forward a plan for utilizing this type of male sterility in a breeding program.

Brar et al. (1980) observed male sterility in crosses between certain cultivars of brown mustard. It would thus seem likely that the sterile individuals observed by Rawat and Anand originated from spontaneous crossings between types of differing cytoplasm. Sterile cytoplasm does not, however, appear to be very common since no genes capable of fully restoring fertility in brown mustard have yet been identified.

By means of backcrossing, Mathias (1985) transferred the genomes of several rape cultivars into the brown mustard cytoplasm discovered by Anand. All the progenies were completely male sterile. The earliest generations produced very little seed, but after two

backcrosses seed production improved.

Rouselle and Eber (1983) noted varying degrees of male sterility in progenies from crosses between brown mustard and rape. Nevertheless, their study did not prove conclusively that the sterility they observed was cytoplasmic in origin.

2.3.2.4 Nig Cytoplasm

Pearson (1972) used the backcrossing technique to transfer the genome of broccoli (B. oleracea) to black mustard (B. nigra) cytoplasm. After certain of the resulting broccoli plants had been pollinated with cabbage, male sterility was observed among the progeny of the hybrids. In the sterile plants, the stamens were reduced to petal- or sepal-like structures, and the nectaries were entirely lacking. This type of male sterility, which occurs only in the presence of the cytoplasm of B. nigra, named nig cytoplasm by Shiga (1980), is brought about by the action of a single recessive gene (p). This gene occurs in fodder kale (B. oleracea) as well as in common cabbage.

In addition to the type of male sterility described above, another less pronounced form could be distinguished in the same material. Apart from having poorly developed stamens, the sterile flowers appear more or less normal. The mode of inheritance of this type of sterility could not, however, be conclusively determined. From the point of view of hybrid production, this latter system might well be preferable to the sterility determined by the p gene, particularly if pollination by insects is essential (Ryder 1979).

So far no mention can be found in the literature of the use of either system in the commercial production of hybrid cultivars.

The cytoplasm of Ethiopian mustard (B. carinata), named car cytoplasm by Shiga (1980), has been shown to resemble nig cytoplasm in some respects (Palmer et al. 1983, Erickson et al. 1983a). Rousselle and Eber (1983) observed that car cytoplasm also gives rise to male sterility in rape.

2.3.2.5. Nap Cytoplasm

The male sterility inducing effect of the nap cytoplasm in rape (B. napus) was observed almost simultaneously in Japan and in England (Shiga and Baba 1971, 1973; Thompson 1972). It is also called Thompson-Shiga cytoplasm after the discoverers. The present, more generally used name of nap was originally proposed by one of the discoverers, Shiga (1980).

Thompson (1972) crossed several winter and spring rape strains with the Polish cultivar Bronowski. Whenever Bronowski was used as the pollinator, some male sterility invariably occurred in F_2. When Bronowski was the female parent in the crosses, no sterility was found. The male sterile plants were characterized by abnormally narrow petals and very short stamens carrying little or no pollen. To account for this behavior, Thompson formulated a hypothesis based on Bronowski being homozygous for a recessive sterility gene (rf) which fails to produce a sterile phenotype in the presence of the cultivar's own cytoplasm (F). Other cultivars and strains have a dominant male fertility gene (RF) set in cytoplasm S, in the presence

of which the effect of the sterility gene is expressed.

Shiga and Baba (1971, 1973) observed male sterility in 23 progenies derived from the cross between the Japanese rape varieties Chisaya-natane and Hokuriku, but only when the former had been used as the female parent. In this case, too, the sterile plants showed narrow petals and short stamens.

On comparing these similar sterile materials originating from different sources, it was concluded by Shiga et al. (1976, 1983) that the male sterility is more likely based on the same kind of cytoplasm in all these cases. Following the proposal of Shiga (1980), the sterile cytoplasm is now termed nap cytoplasm, and the fertile type N cytoplasm.

Shiga et al. (1977), Ohkawa and Shiga (1978) and Ohkawa (1985) examined the progenies from reciprocal crosses between turnip-rape and rape, and studied synthetic rape lines with various cytoplasmic backgrounds. They concluded that the N cytoplasm of B. napus corresponds to the cam cytoplasm of B. campestris from which it was derived. This contention is supported by the results of restriction enzyme analyses (Palmer et al 1983, Erickson et al. 1983a, 1983b, Ohkawa and Uchimiya 1985).

In many respects nap cytoplasm resembles the cytoplasm of cabbage (B. oleracea). It is conceivable that it was originally derived from cabbage, but that it was altered in some degree during the independent specific evolution of rape (Erickson et al. 1983a, 1983b). The normal cytoplasm of cabbage does not cause male sterility in rape (Ohkawa 1985).

The technique of crossing to turnip-rape has been used fairly extensively in rape breeding in Japan, which explains why cam cytoplasm is frequently found in rape cultivars from that country (Shiga 1980). In addition to Bronowski, other European cultivars have revealed fertile N cytoplasm (Shiga 1980), indicating that some backcrossing, intentional or spontaneous, has happened here, too. It is possible that genes causing male sterility have been transferred from turnip-rape to rape (Erickson et al. 1983b). This is confirmed by the more frequent occurrence of male sterility genes among Japanese cultivars than among European ones (Shiga 1976, Shiga et al. 1977, 1983).

The inheritance of fertility restoration in nap cytoplasm has been investigated by many workers. Rouselle and Renard (1978, 1979) crossed 20 varieties of rape with Bronowski. The segregation ratios in F_2 indicated that the expression of male fertility is controlled by two pairs of alleles. Sernyk (1982) found the Canadian cultivar Regent to possess at four or five loci genes influencing fertility restoration. At each locus the allele causing male sterility was recessive. In crossed material comprising 131 Japanese and 68 European strains and cultivars, it was found that depending upon the particular crossing, the restoration of fertility required between one and four dominant genes (Shiga 1976, Shiga et al. 1983).

Both the cam and the nap types of cytoplasm also occur naturally in B. campestris, though the cam type is more common. The nap cytoplasm is encountered mainly in more turnip-like cultivars (Erickson et al. 1983a, Ohkawa 1981, 1985, Ohkawa and Shiga 1981,

Palmer et al. 1983). The sterile cytoplasm originally observed by Ohkawa (1981) in a wild turnip from New Zealand and which was named 1-4, has proved identical to nap cytoplasm (Ohkawa 1983, 1985; Ohkawa and Uchimiya 1985). The influence of nap cytoplasm on the morphology, and particularly on the male fertility of turnip-rape is similar to its influence upon rape (Ohkawa 1985).

Of the thirty-five strains of B. campestris studied by Ohkawa (1985), six were found to have no fertility restoring genes. At present it appears to be uncertain how many pairs of alleles determine fertility in turnip-rape, considering the interaction with nap cytoplasm.

The main obstacle to the utilization of nap cytoplasm in the hybrid breeding of Brassica species appears to be the poor stability of the sterility conferred by nap. Several observations have indicated that at high temperatures sterile plants begin to produce pollen. Fan and Stefansson (1986) noticed this happening in rape (B. napus) when the day temperature rose above 26°C. At a temperature of 22°C no pollen formation was observed. In Chinese cabbage (B. campestris ssp. pekinensis) pollen formation began at 20°C (Ohkawa and Shiga 1981). Sterile plants frequently start to produce pollen towards the end of their flowering period (Shiga and Baba 1973, Ohkawa and Shiga 1981, Ohkawa 1985). For these reasons the nap system is generally regarded, with certain exceptions, as being too unstable for use in commercial hybrid seed production.

2.3.2.6 Pol Cytoplasm

At the time of writing there appears to be no definite information concerning the origin of pol cytoplasm, although it seems at least possible that B. juncea is the primary source of this type of cytoplasm (Erickson et al. 1986). Fu (1981) recorded an observation made in China of cytoplasmically inherited male sterility in the Polish B. napus cultivar 'Bo-Li-Ma', for which the name 'Polima' has also been used. On the basis of this observation and according to the convention proposed by Shiga (1980), this type of cytoplasm is now generally known by the abbreviation pol (Fan 1985). In some respects pol cytoplasm is similar to nap cytoplasm (Fan 1985). In both cases sterility results from failure of the archesporia to differentiate. As with nap cytoplasm, the pol type fertility tends to be restored at high temperatures, although in the latter case considerably higher temperatures appear to be needed to break down the sterility. According to Fan and Stefansson (1986), pollen formation starts in pol male sterile rape when day temperatures reach 30°C, while with nap cytoplasm some pollen is formed at 26°C.

Fan et al. (1986) also point out that the genes capable of restoring fertility within the nap system do not have the corresponding effect in the case of the pol system. Until now no published results have appeared on genes of B. napus or B. campestris capable of fully restoring fertility within the pol system. Fertility restoration for pol cytoplasm, however, seems to be available in B. juncea (Fan et al. 1986).

Partial restorer genes for pol cytoplasm has been found in the B. napus winter rape cultivar "Italy". This has allowed Canadian rapeseed breeders to use this CMS system for producing hybrid B. napus seed in the field. (McVetty et al. 1986). The same authors also believe that the hybrid B. napus canola cultivars presently in trials are "only the first wave of canola hybrids to be developed in Canada", soon to be followed by B. campestris hybrids and special quality B. napus hybrids.

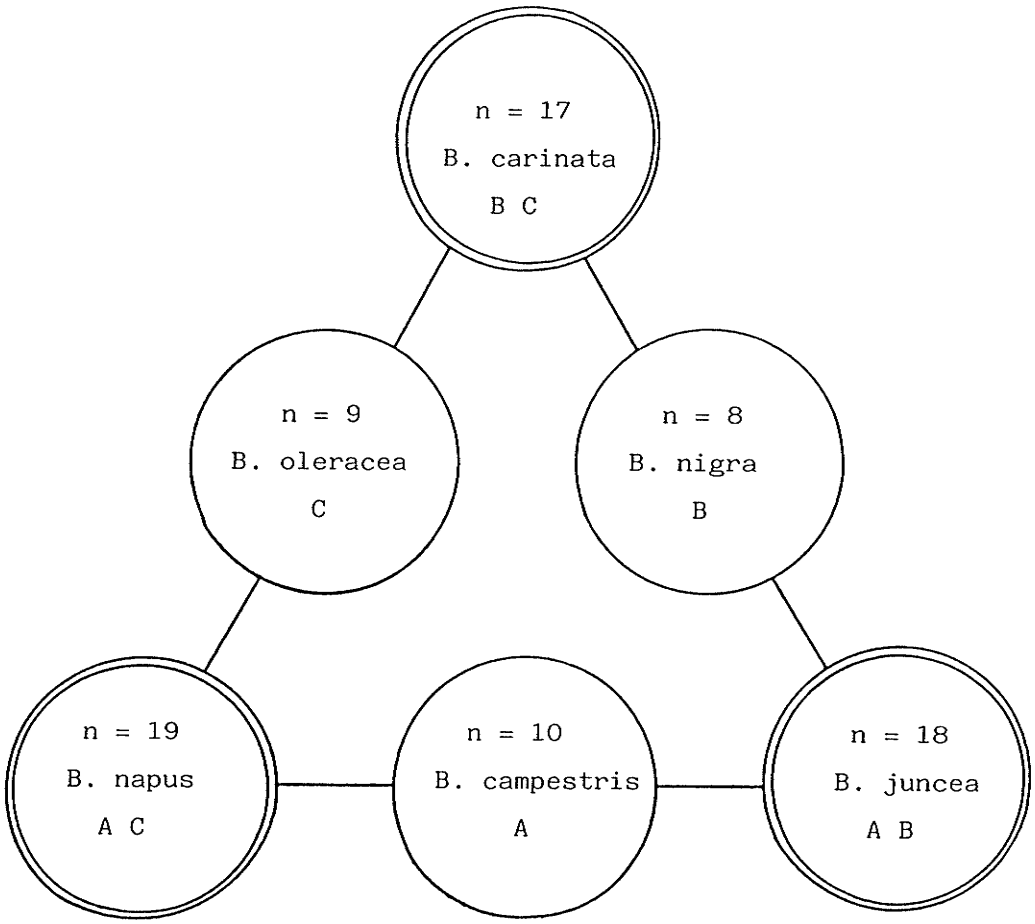


Figure 2.1 The triangle of U illustrating the relations between six species of genus Brassica (modified from U 1935).

III CHAPTER

THE EFFECT OF STERILITY INDUCING CYTOPLASMS ON THE MALE FERTILITY OF THE F_1 PROGENY OF BRASSICA NAPUS L. X B. CAMPESTRIS L. HYBRIDS

3.1 Abstract

Male sterility of the F_1 progenies from interspecific crosses between male sterile lines of Brassica napus L. with cytoplasms mur, nap, ogu or pol and strains of Brassica campestris L. was investigated at Winnipeg, Canada and Jokioinen, Finland. All progenies with ogu cytoplasm were male sterile. Progenies with mur cytoplasm were mostly fertile, but a few sterile plants were observed. None of the F_1 progenies from crosses involving nap cytoplasm was completely sterile. An interaction between environment and the expression of male sterility in nap cytoplasm was observed. Both sterile and fertile progenies were observed in the F_1 generation of crosses involving pol cytoplasm. The B. campestris strains of Chinese origin produced predominantly sterile F_1 progenies while those of Swedish origin produced a high frequency of completely fertile progenies.

3.2 Introduction

Reports of substantial heterosis for seed yield in F_1 hybrids in oilseed rape, B. napus and B. campestris (Hutcheson et al. 1981, Lefort-Buson and Dattee 1982, Sernyk and Stefansson 1982) have stimulated interest in hybrid breeding. Commercial production of hybrid cultivars is, however, possible only if a suitable system for pollination control is available.

Self-incompatibility or possibly male gametocides could be used to produce hybrid seed. However, cytoplasmic male sterility (CMS) has been used successfully in many crops and probably offers the best prospects for use in hybrids of Brassica oilseed crops. Several CMS systems have been reported in the genus Brassica including those based on mur, nap, ogu and pol cytoplasms.

The first type of cytoplasm observed to cause male sterility in crucifers was discovered in an unidentified Japanese cultivar of radish (Raphanus sativus) by Ogura (1968). This cytoplasm is known as the ogu cytoplasm (Shiga 1980). The genomes of several species of Brassicaceae, including various types of B. napus, B. campestris and B. oleracea have been introduced into ogu cytoplasm by means of repeated backcrosses (Bannerot et al. 1974, Williams and Heyn 1981). Some chlorosis usually occurs in these male sterile plants especially under cool conditions (Bannerot et al. 1977). No fertility restorers have been found in these species of Brassica (Bannerot et al. 1977, Williams and Heyn 1981, Rousselle 1982, Fan and Stefansson 1986). Introgression of the fertility restoring system from R. sativus to B. napus was reported by Heyn 1978. However, this report has not been

confirmed, and ogu cytoplasm cannot be used to produce hybrid cultivars of oilseed rape until a restorer is available.

Cytoplasmic male sterility in cultivars of B. napus was discovered independently in Japan and England (Shiga and Baba 1971, Thompson 1972). The male sterility inducing cytoplasms from both sources were later shown to be identical (Shiga et al. 1976). Shiga (1980) proposed the name nap for this cytoplasm. This name is now generally accepted. Shiga (1980) also indicated that the fertile cytoplasm (N cytoplasm) found in many cultivars of B. napus is the same as the cam cytoplasm often found in cultivars of B. campestris. The results from certain interspecific crosses between B. napus and B. campestris (Shiga et al. 1977, Ohkawa and Shiga 1978, Ohkawa 1985) and studies with synthetic lines of B. napus support this hypothesis.

There are many restorers and few maintainers for the nap cytoplasm in B. napus. Up to five dominant genes restore fertility in this cytoplasm (Shiga 1976, Shiga et al. 1983, Ohkawa 1981, Ohkawa and Shiga 1983). Both maintainer and restorer genes for the nap cytoplasm occur in B. campestris (Shiga et al. 1977, Ohkawa and Shiga 1978, Ohkawa 1985).

The sterility of male sterile plants with nap cytoplasm is usually stable at low temperatures. However, Ohkawa and Shiga (1981) observed pollen development in male sterile Chinese cabbage (B. campestris ssp. pekinensis) at a temperature of 20°C and Fan and Stefansson (1986) observed pollen development in oilseed B. napus at 26°C. The male sterile plants also tend to produce some pollen towards the end of the flowering period (Shiga and Baba 1973, Ohkawa

1981). The occurrence of few maintainers and many restorers as well as the temperature sensitivity of the male sterility trait mitigate against the use of nap cytoplasm in commercial hybrid cultivars.

Cytoplasmic male sterility has been reported in plants of the cultivar of B. napus "Bo Li Ma" from Poland (Fu 1981). The name Polima has also been used for this cultivar and the cytoplasm has been named pol cytoplasm (Fan 1985).

The stamen ontogeny is similar for the male sterile plants with either nap or pol cytoplasm (Fan 1985). Fan and Stefansson (1986) observed that at high temperatures the sterility of the pol male sterile plants is more stable than that of nap male steriles, although some pollen formation was observed in pol male steriles at 30°C. There are many maintainers or partial maintainers for the pol cytoplasm in B. napus. Strains that restore fertility to nap cytoplasm do not usually restore fertility to pol cytoplasm. Thus the two cytoplasms are distinct (Fan and Stefansson 1986).

Shiga (1980) proposed the name mur for the cytoplasm of the sand rocket (Diplotaxis muralis (L.) DC). This cytoplasm induces male sterility in some strains of B. campestris while fertility restorers are also available in B. campestris (Hinata and Konno 1979). Fan et al. (1985) discovered that the sterility in B. napus with mur cytoplasm was associated with an extra chromosome, probably of D. muralis origin.

The F_1 progeny of crosses between B. napus and B. campestris have been used to investigate the relationship between the cytoplasms found in the two species (Shiga et al. 1977, Ohkawa 1978, Ohkawa

1985). These studies indicated that maintainers and restorers for the nap cytoplasm in B. campestris are also expressed in interspecific hybrids between the two species. It was assumed, when planning for this study, that restorers and maintainers for the other cytoplasms would be expressed in a similar way.

The purpose of the present investigation was to obtain information that would be useful in developing hybrid cultivars in B. campestris using any of the four (mur, nap, ogu and pol) male sterility inducing cytoplasms. This includes information on the ability of different strains and cultivars of B. campestris to act as both maintainers and restorers for these cytoplasms.

3.3 Materials and Methods

Since male sterile lines or populations in B. campestris were not available when this project was initiated, male sterile lines of B. napus with each of the four cytoplasms (Table 3.1) were used as the female parents in crosses with a number of strains of B. campestris. These crosses are easily made and in the F_1 flower and anther development appears to be normal with abundant pollen shed (personal communication, B. R. Stefansson). To obtain evidence that male sterility was cytoplasmic rather than genetic, some crosses were also made to the nap maintainer with the fertile cytoplasm designated N by Shiga. This cytoplasm is also known as cam cytoplasm. The F_1 progenies of these crosses were used to obtain information on the occurrence of maintainer and restorer genes for the four cytoplasms in B. campestris.

Regent was used as the recurrent parent in the development of male sterile lines of B. napus in several different cytoplasms (Table 3.1). The male parents (B. campestris) which were chosen from different sources are listed in Table 3.4.

The crosses were made during the spring and summer of 1983 in the greenhouse of the Department of Plant Science, the University of Manitoba. The plants that required vernalization were first grown in the greenhouse for four weeks after which they were moved for two months to an illuminated cold storage. The temperature in the storage chamber was approximately 2°C. Greenhouse temperatures varied between 15°C and 35°C but were mostly around 20°C - 25°C.

Ten individuals were grown of each pollinating strain or cultivar. Five of these were chosen at random and crossed with the sterile B. napus lines. One of these plants was also crossed with the fertile nap maintainer line MSUM. The development of the pods was observed and the crosses were repeated when necessary. The pollen parents were cut back and fertilized regularly in order to prolong the flowering period. For this reason new crosses could be made continuously for several weeks after the first ones.

The program involving mur cytoplasm could not be completed as planned because only ten B. napus (mur) individuals out of a total of 120 grown were completely sterile. All individuals with nap, ogu and pol cytoplasm were highly sterile and none had to be discarded.

The F_1 progenies were grown in a field nursery at the University of Manitoba in Winnipeg during the summer of 1984. The material was sown May 30th to June 1st in randomized order. The test was not

replicated because the main purpose of the study was to examine differences between the cytoplasms and groups of B. campestris strains and cultivars rather than the differences between the individual parents. One row, approximately 45 cm long was sown, of each F_1 progeny. The number of seeds sown per plot varied between twenty and fifty depending on the condition (seed size, germinated seed, shrivelled seed) of the seeds. A space of 10 cm was left between the ends of the plots. The space between the rows was 25 cm. After emergence, the plots were thinned to leave about twenty plants per plot. Two rows of B. campestris cultivar Tobin were sown on the edges of the experiment to protect it from border effects.

A number of plots were lost because of heavy rains and flooding in early June.

The level of male sterility of individual plants was observed in each plot when the whole plot was flowering. In each progeny the anther development and pollen shed of the ten first plants in the row was evaluated. The individuals that did not release any visible amounts of pollen were classified as 1 (completely sterile). Those that had visibly deformed anthers with some pollen shed were classified as 2 (partially sterile). Plants with normal or nearly normal anthers and abundant pollen shed were classified as 3 (completely fertile).

On the basis of these data the F_1 progenies were arranged into three classes: completely sterile, with all observed individuals completely male sterile; intermediate or segregating, with all individuals neither completely male sterile nor completely fertile;

and completely fertile. The first observations were made July 12th and the last ones July 30th. The plots that had not come into flower by the last date were not evaluated, since changes in the weather might have made it impossible to compare the observations with the earlier ones. Many of the late flowering plants were also badly injured by insects which would also have made the comparison difficult. At Winnipeg the average temperatures in June and July were 17.0°C and 19.6°C, respectively and the daily maximum temperatures were often over 30°C.

During the summer of 1985 those F_1 progenies of which there still remained seed, were studied in the field in Jokioinen. The experiment was sown May 20th to May 21th. The field arrangement was similar to the one in Winnipeg, except that the length of the rows was one meter. The method of observation was the same as used in Winnipeg, the first observations being made July 8th and the last on July 27th. The progenies that had not flowered by that time had to be discarded because their buds were almost totally destroyed by the pollen beetle (Meligethes aeneus Fabr.). The plots could not be sprayed because of pollinating insects in nearby trials. Average temperatures for June and July were 13.2°C and 15.3°C. Temperatures over 20°C were measured on 27 days between June 6th and July 27th but only on three days during late June did the maximum exceed 26°C.

The numbers of plots sown and observed in Winnipeg and Jokioinen are given in Table 3.2. Statistical analyses were made using the chi-square test to compare the frequencies of F_1 progeny classes completely male sterile intermediate or segregating and completely

fertile between sites and within cytoplasms. In order to compare cytoplasms or locations the progenies were arranged in pairs with a common male parent (cytoplasms) or common origin (locations). Progenies without a counterpart were not used. A SPSS-X statistical package in a VAX 11-780 computer was used for sorting the data.

3.4 Results and Discussion

3.4.1 N Cytoplasm

All 31 progenies with N cytoplasm that were observed in Winnipeg in the summer of 1984 had well developed anthers and produced an abundance of pollen. These progenies (n=29) which were derived from crosses between a tetraploid (n=19) and a diploid (n=10) species were phenotypically quite similar to one another. They showed strong dominance of B. napus-like characters in most respects.All plants observed produced some seed.

3.4.2 Mur Cytoplasm

The results of the crosses B. napus (mur) x B. campestris are presented in Table 3.3. Sterility was observed in three cross progenies with mur cytoplasm. In each of these progenies completely fertile individuals were also observed. Sterility was frequently expressed in a mosaic fashion in that many of the plants with male sterile flowers also bore male fertile flowers. In some cases both sterile and fertile anthers were found in the same flower. This mosaicism might result from instability caused by the extra chromosome which Fan et al. (1985) found to be associated with male

sterility in the B. napus (mur) population used as the female parent in the present crosses.

3.4.3 Ogu Cytoplasm

In Winnipeg, 181 progeny groups derived from crosses between male sterile B. napus with ogu cytoplasm and 41 strains of B. campestris were observed. They were all completely male sterile (Table 3.4) with no indication of pollen development. Male sterility was also complete in Jokioinen where 87 progeny groups representing twenty strains were observed.

No fertility restorers for ogu cytoplasm have been reported in any Brassica species studied so far (Rousselle 1979, Williams and Heyn 1981, Leung and Williams 1983, Fan et al. 1986). Thus, the results of the present study are in agreement with previous observations.

The sterility in the F_1 progenies with ogu cytoplasm is complete and stable and the problems (chlorosis, lack of nectaries) connected with the cytoplasm (Rousselle 1979, 1982) can now be solved using protoplast fusion (Pelletier et al. 1983). It is likely that ogu cytoplasm could be used for hybrid production in B. campestris. The utilization in hybrid breeding of the oilseed forms is, however, feasible only when restoration of fertility becomes available in this species.

3.4.4 Nap and Pol Cytoplasms

The observations on the fertility of the crossing progeny groups are summarized in Table 3.4. No completely sterile F_1 progenies were found with nap cytoplasm and, on an individual plant basis, only four hybrids could be classifed as completely male sterile. Thirty individuals were classified as partially sterile. These individuals were distributed among fourteen F_1 progenies. Many of the progenies in which partial sterility was observed arose from crosses with strains of Indian origin. Also a fairly high frequency of partial sterile individuals were noted in the progenies of some Chinese strains.

Most hybrids with pol cytoplasm were sterile or partially sterile. A total of 57 F_1 progenies were sterile, 106 progenies were classified intermediate or segregating, while only 18 were completely or almost completely fertile. The variability in the level of sterility among the progenies obtained from crosses with different individuals of the same strain indicated that many strains of B. campestris are heterogeneous with respect to fertility restoration in pol cytoplasm.

The occurrence of maintainers and restorers for pol cytoplasm in B. campestris according to geographical areas of origin of cultivars was studied using the data from the material grown in Winnipeg. The results can be seen in Table 3.5. The cultivar R-500 from Canada, a good maintainer for pol cytoplasm, was omitted from the comparison because it is clearly different from the rest of the Canadian cultivars, both in type and origin (Anon. 1984). Fertile,

intermediate and sterile progenies could be found in each group, but the frequencies differed greatly from group to group.

The highest frequency of fertile F_1 progenies was found among the progenies of the Swedish strains and cultivars, eg. cultivars Rapido I and Torpe appeared to be good restorers for pol cytoplasm. The Chinese material produced predominantly sterile progenies and two strains of Chinese vegetable type B. campestris appeared to be complete maintainers. A high frequency of male steriles was also observed in the progenies of the Indian cultivars although most progenies in this case were intermediate. In particular the cultivars BSH-1 and Pusa Kalyani appeared to be good maintainers for pol cytoplasm. Canadian cultivars produced mainly intermediate progenies although both fertile and sterile progenies were also observed.

In Jokioinen 84 progenies with nap cytoplasm and 89 progenies with pol cytoplasm were observed. Difference between cytoplasms was clearly expressed at this location, though not as pronounced as in Winnipeg. In the pol group 27 progenies were sterile and 59 intermediate or segregating. The observations on the abilities of different strains and cultivars to maintain sterility or restore fertility in pol cytoplasm agreed well with the observations made in Winnipeg.

No sterile F_1 progenies were observed in the nap group, although 29 expressed an intermediate reaction. In addition to those found to be partial maintainers in Winnipeg, the cultivars Span and Tobin from Canada, as well as strains Sv 68-420 from Sweden and TL-15 from India, were also observed to maintain partial sterility in nap cytoplasm.

A direct comparison between the cytoplasms at both locations can be seen in Table 3.6. Only those progenies with the half-sib in the other cytoplasm have been included. The difference between the cytoplasms is evident from these results. No sterile progenies with nap cytoplasm were observed at either location while the frequency of sterile progenies with pol cytoplasm is high at both Winnipeg (47 out of 149) and at Jokioinen (25 out of 82).

Omitting all the progenies that were not observed at both locations, a comparison between the two environments was made using contingency X^2 test on 2 x 2 (nap) and 2 x 3 (pol) tables. The results are given in Table 3.7. The distribution of the progeny groups with nap cytoplasm in different fertility classes was significantly different in Winnipeg and Jokioinen (X^2 = 21.4***, d.f. 1). Only 4 progenies were classified as intermediate in Winnipeg, and 24 in Jokioinen. The total number of progenies with nap cytoplasm was 76 at both locations. For the pol cytoplasm group the difference was not significant (X^2 = 1.9, N.S. d.f. 2) although the number of completely fertile progenies was lower at Jokioinen than at Winnipeg.

On the basis of these data it seems likely that the cytoplasms in question react differently to different environments and the sterility induced by pol seems to be more stable than that obtained with nap cytoplasm. The effect of temperature on the fertility of plants with nap cytoplasm has been reported by Ohkawa and Shiga (1981) as well as Fan and Stefansson (1986). The latter workers also found that pol cytoplasm is less sensitive in this respect than nap cytoplasm. It is plausible therefore that differences in temperatures at least partially explain the differences in fertility of the nap

material at Winnipeg and Jokioinen. The day-temperatures before and during the flowering of the observed material at Winnipeg were considerably higher than at Jokioinen.

The results of this study indicate that pol cytoplasm is potentially useful in the hybrid breeding of B. campestris. Both maintainers and restorers for pol seem to be available in the oilseed type cultivars of B. campestris. Suitable lines or populations for developing hybrid cultivars could be selected from the material presently in cultivation and no genes would need to be introduced from other species or subspecies.

The present results with nap cytoplasm differ somewhat from those of Shiga et al. (1977) and Ohkawa (1985), who reported complete sterility in progenies from interspecific crosses of male sterile B. napus (nap) and different types of B. campestris. It should be taken into consideration, however, that the strains and cultivars used in their studies were different from the material used in this study. In previous studies the development of the stamens rather than just pollen production in the anthers was considered. These differences together with the different environmental conditions may account for the differences in the results.

Ohkawa (1985) proposed that nap cytoplasm could be used for F_1 seed production in B. campestris crops, especially vegetables. On the basis of the results of the present study it may be concluded, that although this may be possible with some crops under certain circumstances, the prospects for using nap cytoplasm for hybrid production in oilseed B. campestris in Canada or North Europe are not as good as the prospects for the use of the pol cytoplasm.

TABLE 3.1.

Male sterile and male fertile strains of *Brassica napus* used as female parents in crosses with strains of *Brassica campestris*

Cytoplasm	Origin of the Cytoplasm	Description of the Populations
mur	*Diplotaxis muralis*	*D. muralis* x *B. napus*, BC_4
N (fertile)	*B. napus*	MSUM *nap* maintainer, selection from BC_2 progeny of Bronowski x Regent
nap	*B. napus*	Regent x MSUM, BC_2
ogu	*Raphanus sativus*	French *B. napus* (*ogu*) x Regent, BC_2
pol	*B. napus*	Polima x Regent, BC_3

TABLE 3.2

The F_1 progenies from crosses between individual plants of *B. napus* (male sterile, except *N* cytoplasm), and *B. campestris* which were grown at Winnipeg, 1984 and Jokioinen, 1985

Location	Winnipeg					Jokioinen		
Cytoplasm of the female parent	*mur*	*N*	*nap*	*ogu*	*pol*	*nap*	*ogu*	*pol*
No. of strains of *B. campestris* used as male parents	7	41	41	41	41	20	20	20
No. of rows of F_1 progenies observed in field	15	31	162	181	182	84	87	89

TABLE 3.3

Frequency of male sterility and fertility in the F_1 generation of crosses involving male sterile B. napus (mur) and strains of B. campestris

Strains or Cultivar	Male Sterility of Progeny Groups from Individual Crosses			Male Sterility of Plants within Crosses	
	No.of Progenies	Inter-mediate	Fertile	Sterile	Fertile
BSH-1	3	0	3	0	30
12	2	0	2	0	20
15	2	0	2	0	20
26	2	0	2	0	20
40	2	1	1	1	19
41	3	2	1	6	24
49	1	0	1	0	10
Total	15	3	12	7	143

TABLE 3.4

Male fertility or sterility of F_1 hybrid progenies from crosses involving male sterile B. napus with nap, ogu and pol cytoplasms and strains of B. campestris, Winnipeg 1984.

Strain or Cultivar	Type	Country of Origin	Type of the F_1 Progenies*		
			nap	ogu	pol
10	spring oilseed	Afghanistan	F, I	S	F, S
Bird Rape	weed	Canada	F	S	F, I
R-500	yellow sarson	Canada	F	S	I, S
Span	spring oilseed	Canada	F	S	F, I
Tobin	Canola	Canada	F	S	I, S
Torch	spring oilseed	Canada	F	S	I
Ravizzone	winter oilseed	Chile	F, I	S	F, I
Golden	spring oilseed	China	F	S	I, S
Hubei Youcai	vegetable	China	F, I	S	F, I, S
Qingyou Wuhao	spring oilseed	China	F	S	I, S
12	vegetable	China	F, I	S	I, S
23	vegetable	China	F	S	S
26	winter oilseed	China	F, I	S	I
40	winter oilseed	China	F, I	S	I, S
41	vegetable	China	F	S	I, S
44	vegetable	China	F	S	I, S
45	vegetable	China	F	S	I, S
46	vegetable	China	F	S	S
BSH-1	brown sarson	India	F	S	I, S
IARI	yellow sarson	India	F	S	I
ITSA	toria	India	F, I	S	I, S
Pusa Kalyani	brown sarson	India	F, I	S	I, S
TL-15	toria	India	F, I	S	I, S
Toria-7	toria	India	F, I	S	I, S
47	toria	India	F, I	S	I, S
48	toria	India	F	S	I, S
49	toria	India	F	S	F, I
36	toria	Nepal	F	S	I, S
K-645	toria	Pakistan	F, I	S	I, S
K-660	toria	Pakistan	F	S	I, S
15	spring oilseed	Poland	F	S	I, S
9	weed	Puerto Rico	F	S	F, I
19	weed	Spain	F	S	F, I
Rapido I	winter oilseed	Sweden	F	S	F, I
Sv03202	Canola	Sweden	F	S	F, I
Sv68-420	spring oilseed	Sweden	F	S	I
Sv7510087	spring oilseed	Sweden	F, I	S	F, I, S
Torpe	spring oilseed	Sweden	F	S	F, I
70-9558	spring oilseed	Sweden	F	S	F, I
1	vegetable	Thailand	F	S	S
6	spring oilseed	Turkey	F	S	I, S

* F, fertile; I, intermediate or segregating; S, sterile

TABLE 3.5

Frequencies of male sterile, intermediate and fertile progeny groups in the F_1 generation of crosses involving male sterile *B. napus* with *pol* cytoplasm and strains of *B. campestris* from different regions, Winnipeg 1984.

Region of Origin	No. of Crosses	Male Sterility of the Progeny Groups		
		Sterile	Intermediate	Fertile
India, Nepal or Pakistan	55	19	35	1
Canada	17	1	13	3
China	47	27	19	1
Sweden	29	1	20	8
Other	30	8	17	5
Total	178	56	104	18

TABLE 3.6

Comparison between frequencies of male sterile and male fertile progenies in the F_1 generation of crosses involving male sterile *B. napus* with *nap* and *pol* cytoplasm and strains of *B. campestris*.

	Winnipeg		Jokioinen	
Type of Progeny Group	*nap*	*pol*	*nap*	*pol*
Sterile	0	47	0	25
Intermediate	11	86	28	54
Fertile	138	16	54	3
Total	149	149	82	82

TABLE 3.7

Comparison of frequencies of male sterile and male fertile progeny groups in the F_1 generation of crosses involving male sterile *B. napus* with *nap* and *pol* cytoplasm and strains of *B. campestris*. Comparison between the two locations, Winnipeg and Jokioinen.

Type of Progeny Group	Cytoplasm of female parent					
	nap			*pol*		
	Winn.	Jok.	total	Winn.	Jok.	total
Sterile	0	0	0	25	29	54
Intermediate	4	27	31	54	54	108
Fertile	72	49	121	7	3	10
Total	76	76	152	86	86	172

X^2 (nap) = 21.4 *** d.f. 1
X^2 (pol) = 1.9 N.S. d.f. 2

IV CHAPTER

OBSERVATIONS ON THE EFFECTS OF *POL* CYTOPLASM IN *BRASSICA CAMPESTRIS* L. IN A CONTROLLED ENVIRONMENT

4.1 Abstract

Reciprocal crosses were made between four cultivars of *Brassica campestris* L. and fertile individuals of a progeny from a cross between male sterile *B. campestris* (*pol*) and the cultivar Nopsa from Finland. Three pairs of progenies of each cultivar were grown in a controlled environment. Varying frequencies of male sterile plants were observed in the progenies with *pol* cytoplasm while all plants in the progenies with normal cytoplasm were completely fertile. The results support the assumption that the male sterility associated with *pol* cytoplasm is controlled by a cytoplasmic-genic system. Sterility was associated with small petals and slightly irregular growth of the pistils. Sterile plants were also observed to be somewhat taller than the fertile ones. Some completely fertile progenies with *pol* cytoplasm were also observed, which indicates that full restoration of fertility is available in *B. campestris*.

4.2 Introduction

Fu (1981) reported male sterility in plants from a cultivar of Brassica napus "Bo Li Ma" from Poland and suggested a cytoplasmic origin for its sterility. The name "Polima" has also been used for this cultivar and its cytoplasm has been named pol cytoplasm (Fan 1985).

Fan et al. (1986) tested 32 strains of B. napus for their ability to restore fertility in the pol cytoplasm. No good restoration of fertility was found and consequently the inheritance of the fertility restoration could not be studied. Some of the strains appeared to be only partial maintainers. Strains that restored fertility to nap cytoplasm did not restore fertility in pol cytoplasm. These two cytoplasms are consequently distinct. The fertility restoration was derived from crosses between B. napus and B. juncea but was found to be associated with an extra chromosome, probably of B. juncea origin.

Erickson et al. (1986) demonstrated that pol cytoplasm can be distinguished from the other cytoplasms found in B. napus and B. campestris by means of restriction enzyme analysis. The primary source of the cytoplasm was not ascertained, although there was some evidence supporting an origin from B. juncea.

Of the side effects of sterility inducing cytoplasms in Brassica crops, the best known example is the chlorosis caused by the ogu cytoplasm from radish, first reported by Bannerot et al. (1977). This problem is associated with the interaction between Raphanus chloroplasts and the Brassica nucleus (Pelletier 1983).

Shiga (1976) reported that male sterile plants with nap cytoplasm were slightly shorter than fertile plants and had small and rugose petals. Small petals are also associated with the pol sterility in B. napus (Fu 1981).

Both cam and nap cytoplasms are found in B. campestris (Shiga 1980) although cam cytoplasm is more common. For this reason and because nap cytoplasm has mainly been found in more turnip-like strains of B. campestris (Erickson et al. 1983, Ohkawa 1981, Ohkawa and Shiga 1981, Palmer et al. 1983) the cytoplasm of the four B. campestris cultivars used in this study is assumed to be of the cam type although no specific biochemical tests have been made to verify this.

Little information seems to be available concerning the effects of pol cytoplasm in a B. campestris background. The purpose of this study was to gain positive evidence for the maternal inheritance of male sterility associated with pol cytoplasm in B. campestris and to investigate the possible side effects of the cytoplasm and sterility.

4.3 Materials and Methods

The source of the pol cytoplasm for this study consisted of several progenies from crosses between individual plants of B. napus (pol) and B. campestris. A number of strains of B. campestris were used for pollinating completely male sterile plants found in these progenies. Back-crossing was continued with TL-15, a toria-type cultivar from India that seemed to maintain almost complete male sterility and developed rapidly under greenhouse conditions. Sterile

individuals of TL-15 (pol) BC_4 were again pollinated with several cultivars of B. campestris including the Finnish cultivar Nopsa.

The initial work for this study, including the backcrosses with TL-15 and the crosses with Nopsa, was done at the Department of Plant Science, University of Manitoba, in Winnipeg, Canada. The crosses and the experimentation were conducted at the Department of Plant Breeding of the Finnish Agricultural Research Center in Jokioinen, Finland.

Several progenies from the crosses with Nopsa displayed a high level of male fertility. The anthers appeared normal or almost normal, dehiscence was normal and pollen was produced in abundance. The position of the anthers in relation to the stigma was, nevertheless, lower than in the plants with normal cytoplasm. Fertile individuals with pol cytoplasm from one of these cross progenies were crossed reciprocally with individuals of the cultivars Nopsa, R-500 TL-15 and Tobin.

Three pairs of progenies were chosen from each group and twenty plants were grown of each progeny. The plants were placed in a growth chamber in completely randomized design. The seeds were germinated for two days on filter paper in Petri-dishes after which the seedlings were transplanted to 10 cm plastic flower pots filled with homogeneous sand-peatmoss mixture. The photoperiod was 18 h and the temperatures were 20°C by day and 12°C by night. Light was provided by six multimetal lamps, type Osram HQI-T 1000 W/D. Photosynthetic photon flux density at the level of the tops of the plants varied from 290 to 340 uE $m^{-2}s^{-1}$. The plants were watered moderately twice a

day. A liquid horticultural fertilizer was given twice with the water, one week and two weeks after planting.

The development of the anthers and pollen shed were observed two days after the first flowers opened. On the basis of these observations the plants were classified into three male fertility classes: 1. completely male sterile, anthers small, white and conical, apparently no pollen shed; 2. partially male sterile, anthers visibly abnormal, some pollen shed; and 3. male fertile, anthers apparently normal, abundant pollen shed (Fig. 4.1 - 4.4). In addition, the lengths of the stamens and pistils were measured. The base was set at the level of the lower nectaries and the accuracy of the measurements was ± 0.5 mm. These measurement were always made on the youngest completely opened flower of the main raceme while male fertility was evaluated on all the flowers open at the time of observation so that the fertility class of each plant was determined by the most male fertile individual flowers it produced. The height of the plant from soil level to the tips of the buds was measured at the same time and the number of elongated branches was counted. Measurements for the height of the plants were made to the nearest full centimeter. All branches longer than 3 cm were counted and the main raceme was included in the count.

A SPSS-X statistical package in a VAX 11-780 computer was used to process the data. Statistical analyses were made using the F-test and the t-test to compare the variances and averages of the characteristics observed in the progenies of the reciprocal crosses. Since one of assumptions for t-test is that variances of the

populations compared are not significantly different, the t values of the comparisons with significant F values are given in parentheses and should be interpreted with caution as the estimates may be affected by the difference of the variances.

4.4 Results and Discussion

Most of the plants raised were healthy and appeared to develop normally. The germination and early growth in the cross progeny 'R-500 cross 2. (pol)', however, was very slow and uneven and this might account for the amount of variation observed in many of its characters. For this reason the observations on this progeny should be considered with caution.

The project involving reciprocal crosses was designed to test the following hypotheses and assumptions. If the male sterility associated with the pol cytoplasm is actually conditioned by a cytoplasmic-genic system, the fertile pol parent in the reciprocal crosses should be heterozygous for fertility restoration. The progenies with the cam cytoplasm should have normal anther development and pollen production. The progenies with pol cytoplasm should be completely male fertile only if the parent with the cam cytoplasm is homozygous for fertility restoration, otherwise these progenies should segregate for male sterility and male fertility.

The observations on male sterility of the progenies from crosses are summarized in Table 4.1. Widely varying numbers of male sterile plants were observed in the progenies with the pol cytoplasm while no male sterile individuals were observed in the cross progenies with

the cam cytoplasm. This indicates that a maternal inheritance is involved in the expression of the male sterility, and accordingly supports the assumption of a cytoplasmic-genic interaction controlling male sterility in the pol system.

Only one partially sterile individual was found in the progenies of the three crosses involving different individuals of the Finnish cultivar Nopsa as pollinators, which indicates that the male parents in this case were homozygous restorers.

The results with the remaining progenies with pol cytoplasm varied considerably. In most cases segregation for male fertility and male sterility was observed as expected in the case of cytoplasmic-genic inheritance. However, two of the crosses with the Indian cultivar TL-15 as male parent did not produce any male fertile plants in the progeny, which indicates that either the manifestation of the restorer gene(s) is somehow unstable, or that there are some other complications involved in the restoration of male fertility.

The maternal inheritance in the inheritance of stamen length was also clear. All progeny pairs, except two from the crosses with Nopsa, differed significantly with regard to the variance or mean of stamen length. In most cases the amount of variation agreed well with the observed segregation for male sterility (Table 4.2). In many cases, however, the effect of the cytoplasm on the length of stamens is discernible even when only fertile plants are considered. Thus the stamens of the fertile plants in progenies with pol cytoplasm are often significantly shorter than those of individuals in reciprocally crossed progenies. (Table 4.3). This might indicate that the

restoration of fertility can vary in effectiveness even when the anthers, at least under some conditions, look normal. Evidence that restoration can, in some cases, be very nearly complete is afforded from the two pairs of crosses involving Nopsa in which both the averages and distributions of stamen length are very similar in either of the reciprocal crosses.

The differences in stamen length between the male sterile and male fertile plants with pol cytoplasm were tested using the data from the two progeny groups in which both types were represented in every individual cross progeny (Table 4.4). In all tests the stamens of the plants classified as male fertile were significantly longer than stamens of the male sterile or partially male sterile plants.

The size of the petals was also smaller in sterile plants than in fertile ones.This was especially obvious when the petals of the fertile plants with normal cytoplasm were exceptionally large, as the progenies of TL-15 (Fig. 4.5).

Significantly more variation in the length of the pistils was found in the group with pol cytoplasm than in the one with cam cytoplasm (Table 4.5). A significant difference in means could be demonstrated in one cross only. The variability within the pol cytoplasm group seemed to be associated with irregularities in the opening of the flowers, which resulted in crooked pistils that were very difficult to measure accurately. The progenies of cross 3 with R-500 seemed to produce abnormal sepals. In particular, the sterile or partially sterile flowers with small anthers and petals opened very slowly and irregularly. By the time the observations were made,

pistils that normally elongate several days after the flowers have opened, had attained a greater length in sterile (or partially sterile) plants than in fertile plants (Table 4.6).

With respect to flowering time the pol group did not differ significantly from the cam group (Table 4.7). Differences could be observed in two cases only, both with cultivar R-500. In one cross the progeny group with pol cytoplasm was significantly earlier than its reciprocal. Closer examination revealed that this particular family in a background of cam cytoplasm showed a strong tendency to abort its first flower buds, which could delay the start of flowering by several days. There was also a significant difference between the cytoplasms in cross 3 of R-500; whereby the flowers of the pol progeny opened irregularly. In all crosses with Tobin plants classified as male steriles were consistently later than fertile plants with the same cytoplasm and the difference was significant when all three crosses are considered (Table 4.8). In two of the progenies of R-500, however, the fertile plants are significantly later than the sterile ones. Sterile plants were later only in cross 3, which was referred to above, although this difference was not significant. Thus the male sterility itself may not have any effect on the earliness of flowering, but it is possible that there is linkage between the restorer or restorers and a gene(s) contributing to earliness.

The progenies with pol cytoplasm were slightly but significantly taller than the ones with cam cytoplasm (Table 4.9). This tendency was dissernible in most of the individual progeny pairs although the

difference was significant only in cross 1 with R-500. When the male fertile and male sterile plants of the pol progenies of Tobin and R-500 are compared a phenomenon similar to that of the earliness of flowering is seen (Table 4.10). In all three progenies of Tobin the sterile plants are taller than the fertile plants; in two of the progenies the difference was significant. Within the progenies of R-500 the mean height differences between sterile and fertile plants were in opposing directions and only the differences in variances were statistically significant. Because of these discrepancies definite conclusions could not be drawn. However, it seems possible that the male steriles with pol cytoplasm tend to be taller than either the fertiles with the same cytoplasm or the plants with normal cytoplasm. It would be reasonable to assume that earliness and shorter growth are directly associated as shown in the results with the Tobin progenies. The average for the R-500 data supports this hypothesis.

The progenies with pol and cam cytoplasms did not differ with respect to the degree of branching. In two pairs of crosses significant differences were found but they were in opposing directions (Table 4.11). The differences between sterile and fertile plants (Table 4.12), although not significant, are still of biological interest. In all crosses with Tobin the male sterile plants have more branches than the fertiles while in the crosses with R-500 the situation is reversed. These differences were similar to the differences between the two groups in flowering time and plant height.

The data presented supports the hypothesis that male sterility associated with the pol cytoplasm is controlled by a cytoplasmic-genic system. The results also demonstrate that full restoration of fertility is possible in B. campestris. The sterile plants with pol cytoplasm were slightly taller than the fertile ones with cam or pol cytoplasm. No detrimental morphological effects from the pol cytoplasm were observed.

TABLE 4.1.

Male sterility or fertility of the plants in progenies of reciprocal crosses between male fertile B. campestris plants with cytoplasms cam and pol.

	Cam Cytoplasm as Female			Pol Cytoplasm as Female		
	Sterile	Part. sterile	Fertile	Sterile	Part. sterile	Fertile
Nopsa cross 1.*	0	0	20	0	1	19
cross 2.	0	0	20	0	0	20
cross 3.	0	0	20	0	0	20
Tobin cross 1.	0	0	20	7	1	12
cross 2.	0	0	20	6	0	14
cross 3.	0	0	20	2	3	15
TL-15 cross 1.	0	0	20	9	0	11
cross 2.	0	0	20	17	3	0
cross 3.	0	0	20	19	1	0
R-500 cross 1.	0	0	20	7	1	12
cross 2.	0	0	20	3	8	9
cross 3.	0	0	20	4	6	10
Total	0	0	240	74	24	142

* The other parent in these crosses was a heterozygous male fertile B. campestris with pol cytoplasm.

TABLE 4.2

F- and t-tests to compare the effects of cytoplasm (cam or pol) on the differences in stamen length (mm) over reciprocal cross progenies of B. campestris. All plants were included in the comparisons.

	Reciprocal progenies with					
	cam cytopl.		pol cytopl.			
	mean	S.D.	mean	S.D.	F value	t value
Entire population	7.65	0.76	5.69	1.80	5.59***	(15.44***)
Nopsa						
all crosses	7.40	0.66	7.03	0.86	1.69*	(2.67**)
cross 1.	7.30	0.68	6.43	1.10	2.66*	(3.02**)
cross 2.	7.23	0.68	7.10	0.45	2.30	0.69
cross 3.	7.68	0.57	7.55	0.45	1.55	0.77
Tobin						
all crosses	7.28	0.66	5.58	1.50	5.16***	(8.04***)
cross 1.	7.80	0.41	5.75	1.63	15.86***	(5.44***)
cross 2.	7.13	0.63	5.70	1.58	6.35***	(3.76***)
cross 3.	6.93	0.59	5.30	1.30	4.85**	(5.08***)
TL-15						
all crosses	7.93	0.65	3.98	1.68	6.71***	(16.96***)
cross 1.	7.83	0.65	5.45	2.06	9.89***	(4.92***)
cross 2.	7.80	0.70	2.88	0.69	1.03	22.54***
cross 3.	8.15	0.56	3.60	0.62	1.21	24.28***
R-500						
all crosses	7.98	0.83	6.18	1.53	3.42***	(8.00***)
cross 1.	8.15	0.61	5.98	1.52	6.20***	(5.95***)
cross 2.	8.25	0.60	6.48	1.59	7.07***	(4.69***)
cross 3.	7.53	1.03	6.10	1.51	2.14	3.48***

TABLE 4.3

F- and t-tests to compare the effects of cytoplasm (*cam* or *pol*) on the differences in stamen length (mm) over reciprocal cross progenies of *B. campestris*. Only fertile plants were included in the comparisons.

	Reciprocal progenies with					
	cam cytopl.		*pol* cytopl.			
	mean	S.D.	mean	S.D.	F value	t value
Entire population	7.55	0.78	6.94	0.89	1.31	6.28***
Nopsa						
all crosses	7.40	0.66	7.08	0.77	1.35	2.46**
cross 1.	7.30	0.68	6.55	0.97	2.06	2.78**
cross 2.	7.23	0.68	7.10	0.45	2.30	0.69
cross 3.	7.58	0.57	7.55	0.46	1.55	0.77
Tobin						
all crosses	7.28	0.66	6.43	0.87	1.74	5.34***
cross 1.	7.80	0.41	6.88	0.77	3.54**	(3.84***)
cross 2.	7.13	0.63	6.64	0.60	1.08	2.26*
cross 3.	6.93	0.59	5.87	0.90	2.30	3.97***
R-500						
all crosses	7.98	0.83	7.37	0.84	1.03	3.28
cross 1.	8.15	0.61	7.13	0.53	1.33	5.02***
cross 2.	8.25	0.60	7.72	1.33	4.95**	(1.14)
cross 3.	7.53	1.03	7.35	0.47	4.73*	(0.64)

TABLE 4.4

F- and t-tests for the differences in stamen length (mm) between totally or partially male sterile and male fertile plants of *B. campestris* with *pol* cytoplasm

	Completely or partially sterile		Fertile			
	mean	S.D.	mean	S.D.	F value	t value
Entire population	4.46	1.05	6.83	0.97	1.16	12.54***
Tobin						
all crosses	3.76	0.75	6.43	0.87	1.34	12.13***
cross 1.	4.06	0.94	6.88	0.77	1.49	7.01***
cross 2.	3.50	0.45	6.64	0.60	1.81	12.91***
cross 3.	3.60	0.65	5.87	0.90	1.89	6.09***
R-500						
all crosses	4.91	0.96	7.37	0.84	1.33	10.51***
cross 1.	4.25	0.38	7.13	0.53	1.95	14.19***
cross 2.	5.45	0.91	7.72	1.33	2.14	4.36***
cross 3.	4.85	1.06	7.35	0.47	4.95*	(6.83***)

TABLE 4.5

F- and t-tests to compare the effects of cytoplasm (cam or pol) on the differences in pistil length (mm) over reciprocal cross progenies of B. campestris. All plants were included in the comparisons.

	Reciprocal progenies with					
	cam cytopl.		pol cytopl.			
	mean	S.D.	mean	S.D.	F value	t value
Entire population	6.63	0.86	6.78	1.01	1.40**	(1.70)
Nopsa						
all crosses	6.31	0.55	6.38	0.75	1.82*	(0.56)
cross 1.	6.28	0.50	6.00	0.71	2.01	1.42
cross 2.	6.08	0.47	6.23	0.53	1.27	0.95
cross 3.	6.58	0.59	6.90	0.70	1.40	1.59
Tobin						
all crosses	6.28	0.64	6.28	0.59	1.17	0.07
cross 1.	6.30	0.44	6.38	0.43	1.08	0.55
cross 2.	6.30	0.88	6.45	0.61	2.12	0.63
cross 3.	6.23	0.55	6.03	0.66	1.44	1.04
TL-15						
all crosses	6.40	0.62	6.58	0.68	1.20	1.48
cross 1.	6.45	0.51	6.63	0.48	1.12	1.11
cross 2.	6.10	0.58	6.63	0.97	2.85*	(2.08*)
cross 3.	6.65	0.65	6.48	0.47	1.90	0.97
R-500						
all crosses	7.55	0.86	7.88	1.05	1.49	1.91
cross 1.	7.58	1.03	7.50	0.76	1.83	0.26
cross 2.	7.70	0.68	7.93	1.32	3.81**	(0.68)
cross 3.	7.38	0.83	8.23	0.90	1.13	3.09**

TABLE 4.6

F- and t-tests for the differences in pistil length (mm) between totally or partially male sterile and male fertile plants of *B. campestris* with *pol* cytoplasm

	Completely or partially sterile		Fertile			
	mean	S.D.	mean	S.D.	F value	t value
Entire population	7.27	1.14	6.96	1.18	1.07	1.45
Tobin						
all crosses	6.26	0.65	6.29	0.57	1.31	0.17
cross 1.	6.50	0.46	6.29	0.40	1.36	1.04
cross 2.	6.42	0.49	6.49	0.66	1.82	0.18
cross 3.	5.70	0.84	6.13	0.58	2.07	1.07
R-500						
all crosses	7.93	0.87	7.84	1.20	1.89	0.34
cross 1.	7.25	0.80	7.67	0.72	1.25	1.19
cross 2.	7.91	0.74	7.94	1.86	6.41**	(0.05)
cross 3.	8.50	0.71	7.95	1.01	2.05	1.41

TABLE 4.7

F- and t-tests to compare the effects of cytoplasm (*cam* or *pol*) on the differences in the earliness of flowering (days from sowing to the opening of first flowers) over reciprocal cross progenies of *B. campestris*. All plants were included in the comparisons.

	Reciprocal Progenies with					
	cam cytopl.		*pol* cytopl.			
	mean	S.D.	mean	S.D.	F value	t value
Entire population	31.2	3.3	31.3	3.7	1.20	0.31
Nopsa						
all crosses	30.2	3.4	30.4	3.9	1.26	0.33
cross 1.	29.1	2.1	29.1	1.9	1.22	0.08
cross 2.	33.8	2.7	34.9	2.2	1.41	1.42
cross 3.	27.8	2.0	27.3	2.1	1.05	0.77
Tobin						
all crosses	31.4	3.0	31.4	3.5	1.34	0.06
cross 1.	33.8	2.7	32.9	4.3	2.52	0.84
cross 2.	30.8	2.7	31.4	3.4	1.63	0.62
cross 3.	29.5	2.0	29.9	1.9	1.02	0.73
TL-15						
all crosses	29.6	2.2	30.1	2.7	1.56	1.22
cross 1.	28.6	2.2	28.9	3.3	2.19	0.40
cross 2.	30.8	2.1	31.7	2.0	1.09	1.37
cross 3.	29.4	1.6	29.8	2.0	1.44	0.70
R-500						
all crosses	33.7	3.0	33.3	3.7	1.46	0.65
cross 1.	34.7	3.1	30.0	2.9	1.18	4.96***
cross 2.	33.6	2.5	34.5	3.3	1.81	0.97
cross 3.	32.9	3.3	35.5	2.0	2.67*	(2.98**)

TABLE 4.8

F- and t-tests for the differences in the earliness of flowering (days from sowing to the opening of first flowers) between totally or partially male sterile and male fertile B. campestris (pol) plants.

	Completely or partially sterile		Fertile			
	mean	S.D.	mean	S.D.	F value	t value
Entire population	32.9	3.9	32.0	3.6	1.20	1.22
Tobin						
all crosses	33.2	4.1	30.6	2.9	2.00	2.48*
cross 1.	35.0	5.0	31.4	3.2	2.38	1.80
cross 2.	32.7	3.4	30.9	3.4	1.04	1.08
cross 3.	30.8	1.9	29.6	1.9	1.00	1.21
R-500						
all crosses	32.7	3.8	33.9	3.5	1.20	1.32
cross 1.	28.3	2.6	31.3	2.5	1.05	2.41*
cross 2.	32.9	2.4	36.4	3.4	2.03	2.64*
cross 3.	35.9	2.1	35.0	1.9	1.20	0.99

TABLE 4.9

F- and t-tests to compare the effects of cytoplasm (cam or pol) on the differences in the height (cm) of the plants at an early stage of the flowering over reciprocal cross progenies of B. campestris. All plants were included in the comparisons

	Reciprocal progenies with					
	cam cytopl.		pol cytopl.			
	mean	S.D.	mean	S.D.	F value	t value
Entire population	77.0	13.5	80.1	15.1	1.25	2.40*
Nopsa						
all crosses	70.9	10.5	72.5	9.9	1.11	0.86
cross 1.	64.7	11.2	68.3	7.2	2.43	1.23
cross 2.	77.2	9.4	80.1	8.1	1.35	1.04
cross 3.	71.0	6.7	69.2	9.9	2.22	0.64
Tobin						
all crosses	74.2	10.6	77.5	11.0	1.08	1.69
cross 1.	72.4	7.5	74.9	10.8	2.10	0.84
cross 2.	76.7	14.2	81.4	10.9	1.71	1.17
cross 3.	73.6	8.8	76.4	10.7	1.47	0.91
TL-15						
all crosses	74.5	7.0	78.3	11.5	2.66***	(2.22*)
cross 1.	75.6	8.0	80.2	14.5	3.26*	(1.24)
cross 2.	74.9	5.7	78.3	9.7	2.97*	(1.35)
cross 3.	73.0	7.3	76.5	9.8	1.80	1.31
R-500						
all crosses	88.2	17.0	92.0	19.1	1.27	1.14
cross 1.	82.2	13.0	94.3	10.0	1.68	3.31**
cross 2.	94.4	22.2	87.0	21.9	1.03	1.06
cross 3.	88.1	12.6	94.7	22.8	3.27*	(1.13)

TABLE 4.10

F- and t-tests for the differences in the height of plants (cm) at an early stage of flowering between totally or partially male sterile and male fertile plants of B. campestris with pol cytoplasm

Entire population	89.2	16.4	81.8	17.1	1.09	2.36*
Tobin						
all crosses	85.4	9.3	73.9	9.7	1.09	4.41***
cross 1.	83.1	9.8	69.3	7.5	1.70	3.36**
cross 2.	89.8	7.8	77.8	10.1	1.69	2.87*
cross 3.	84.0	10.3	73.9	9.9	1.08	1.93
R-500						
all crosses	91.6	19.5	92.4	19.1	1.03	0.15
cross 1.	89.4	6.6	97.6	10.8	2.67	2.09
cross 2.	90.4	10.3	82.8	31.1	9.20**	(0.70)
cross 3.	94.7	31.8	94.7	9.2	12.08***	(0.00)

TABLE 4.11

F- and t-tests to compare the effects of cytoplasm (cam or pol) on the differences in the number of elongated branches at an early stage of the flowering over reciprocal cross progenies of B. campestris. All plants were included in the comparisons

	Reciprocal progenies with					
	cam cytopl.		pol cytopl.			
	mean	S.D.	mean	S.D.	F value	t value
Entire population	5.3	1.8	5.4	1.6	1.19	0.61
Nopsa						
all crosses	6.0	2.1	5.9	1.9	1.14	0.32
cross 1.	5.3	1.2	5.6	1.6	1.66	0.68
cross 2.	7.9	2.2	7.4	2.0	1.21	0.68
cross 3.	5.0	1.3	4.8	1.1	1.30	0.53
Tobin						
all crosses	5.7	1.5	5.5	1.4	1.21	0.75
cross 1.	6.6	1.3	5.6	1.3	1.00	2.28*
cross 2.	4.9	1.3	5.3	1.6	1.56	0.88
cross 3.	5.8	1.5	5.7	1.3	1.45	0.11
TL-15						
all crosses	4.8	1.2	5.1	1.4	1.52	1.05
cross 1.	4.9	1.5	4.7	1.8	1.44	0.39
cross 2.	4.5	0.8	5.1	1.4	2.76*	(1.81)
cross 3.	5.1	1.1	5.4	1.0	1.02	0.91
R-500						
all crosses	4.6	1.9	5.0	1.6	1.33	1.40
cross 1.	3.3	1.1	4.5	1.5	1.78	2.85**
cross 2.	5.8	1.5	5.3	1.8	1.50	1.06
cross 3.	4.6	2.1	5.3	1.5	1.83	1.22

TABLE 4.12

F- and t-tests for the differences between totally or partially male sterile and male fertile plants of B. campestris (pol) in the number of elongated branches at an early stage of flowering

	Completely or partially sterile		Fertile			
	mean	S.D.	mean	S.D.	F value	t value
Entire population	5.2	1.6	5.3	1.5	1.19	0.21
Tobin						
all crosses	5.9	1.4	5.3	1.4	1.07	1.65
cross 1.	5.9	1.7	5.4	1.0	3.00	0.68
cross 2.	5.7	1.2	5.1	1.7	2.04	0.88
cross 3.	6.4	1.1	5.5	1.2	1.19	1.55
R-500						
all crosses	4.8	1.6	5.3	1.7	1.07	1.19
cross 1.	4.0	1.2	4.8	1.6	1.89	1.31
cross 2.	4.9	1.7	5.7	1.9	1.30	0.92
cross 3.	5.2	1.7	5.4	1.4	1.39	0.29

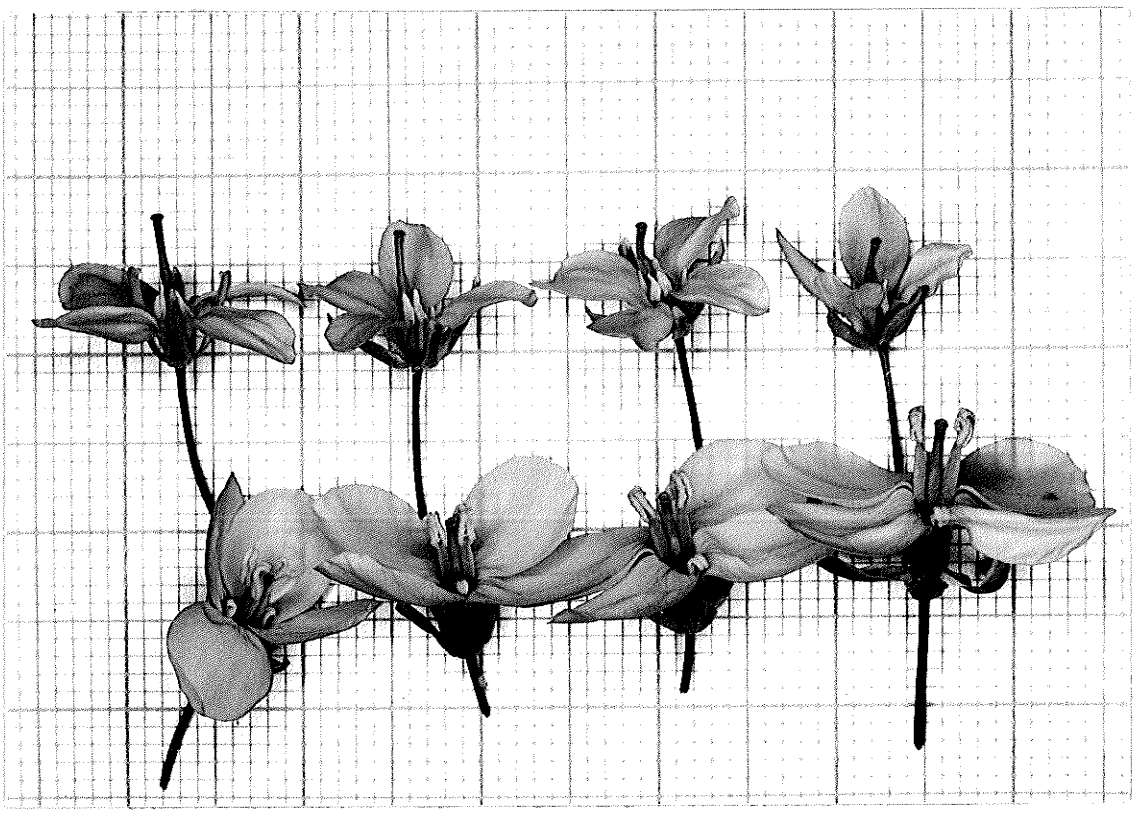
Figure 4.1: Completely male sterile (class 1.) flower of *B. campestris* (*pol*).

Figure 4.2: Partially male sterile (class 2.) flower of *B. campestris* (*pol*)

Figure 4.3: Partially male sterile (class 2.) flower of B. campestris showing pollen shed in one anther

Figure 4.4: Male fertile (class 3.) flower of B. campestris (pol)

Figure 4.5: Flowers of progenies from reciprocal crosses between B. campestris plants with pol and cam cytoplasm. Flowers in the upper row from pol progeny



V CHAPTER

SELECTION OF MAINTAINERS OF MALE STERILITY FOR POL CYTOPLASM IN THREE CULTIVARS OF SUMMER TURNIP-RAPE (BRASSICA CAMPESTRIS L.)

5.1 Abstract

Selection for the maintenance of male sterility in crosses with Brassica campestris L. (pol) was carried out in three B. campestris cultivars. A significant response to selection was observed in all cases. This indicates that heritable variation for maintenance of sterility exists. Evidence for the presence of dominant restorer genes was also observed. The results from the second generation, using selected inbred male parents, indicated that a partially dominant restorer gene may also be involved with the inheritance of fertility.

5.2 Introduction

Considerable heterosis for seed yield has been observed in B. campestris. This can be exploited commercially only if suitable mechanisms for pollination control can be developed.

Sporophytic self-incompatibility is commonly used for producing hybrid cultivars of Brassica vegetables (Ryder 1979). Existing problems with the production and maintenance of the self-incompatible

inbred lines, however, mitigate against its use in oilseed breeding (Hinata and Nishio 1980).

Cytoplasmic-genic male sterility, which in accordance with general convention will be termed cytoplasmic male sterility (CMS), is a system widely used with a number of crops and might be more amenable to manipulation in hybrid breeding of oilseed forms of B. campestris. In this system the gene or genes causing male sterility are expressed only in certain types of cytoplasm. Sterile inbred lines can thus be maintained by crossing with a line that has the same nuclear genotype in different cytoplasm. In order to make hybrid production feasible for crops grown for their seed yield, a dominant restorer gene is required, i.e. a gene that restores fertility in a plant which is otherwise cytoplasmically male sterile.

The effect of pol cytoplasm on male sterility in B. napus L. was first reported by Fu (1981). Fan et al. (1986) examined 32 strains of B. napus in order to find restorers of fertility for pol cytoplasm. Most of the strains were maintainers of complete male sterility. The sterility observed in the progenies of 9 strains was only partial, however, no complete restorers were found in any of the 32 strains. The fertility restoration observed in some progenies derived from interspecific crosses between B. juncea (L.) Czern. and B. napus was found to be associated with an extra chromosome, probably of B. juncea origin. The existence of partial restorers for pol cytoplasm in B. napus has allowed Canadian rapeseed breeders to use this CMS system for producing hybrid B. napus seed in the field (McVetty et al.1986). Fan and Stefansson (1986) observed that the fertility of

male sterile B. napus plants was restored after they had been exposed to 30°C day temperature in a growth chamber.

During the preliminary work done for this project at the University of Manitoba it was observed that crosses between male sterile B. campestris (pol) and several cultivars of B. campestris produced progeny with varying degrees of male sterility. This project was designed to investigate the possibility of selecting maintainers for complete male sterility in pol cytoplasm for use in hybrid production.

5.3 Materials and Methods

The source of pol cytoplasm used in this experiment was a cross between B. napus (pol) and B. campestris, received from Dr. L. Sernyk. Male sterile plants were selected from this cross and backcrossed with Indian cultivar TL-15 that was observed to produce progenies with little or no pollen shed.

The male sterile parents used in the initial crosses of the selection program were individuals of one particular single cross progeny of TL-15 (pol) BC_6 (derived from cross 3, Table 4.1) that had proved completely male sterile. Three cultivars representing three distinct types of B. campestris were used as pollen parents: Nopsa, R-500 and TL-15. Nopsa is a Finnish double low cultivar (low glucosinolate content, practically no erucic acid) that originates from both Swedish and Canadian ancestors; R-500 is a Canadian high erucic cultivar selected from the Indian yellow sarson type of B. campestris; and TL-15 is a toria type cultivar from India. R-500 is

self-compatible while Nopsa and TL-15 are cross pollinating. Both TL-15 and R-500 had previously shown themselves to be fairly good maintainers for pol cytoplasm, while crosses with Nopsa had produced progeny with varying degrees of male sterility in preliminary work.

The procedure for selecting maintainers was as follows. The parents for the crosses, TL-15 (pol) BC_6, TL-15, Nopsa and R-500 were grown in a growth chamber using an 18 h photoperiod and temperatures of 22°C by day and 14°C by night. The development of the anthers and degree of pollen shed of the plants was observed twice, first when flowering started and again two days later. Plants were ranked into three classes according to pollen shed and morphology of the anthers: 1.Completely male sterile with small, white, conical anthers and no pollen shed; 2. partially male sterile with visibly deformed, small anthers and some pollen shed; and 3. male fertile with normal looking fully developed anthers and abundant pollen. Plants were not classified as completely male sterile if pollen was observed in any of the flowers. If most of the flowers produced normal anthers when observed the second time, the plant was classified fertile.

All 140 plants of TL-15 (pol) BC_6 used in this project were classified as completely sterile. The male parents were crossed with the male sterile plants with pol cytoplasm and were also selfed.

The seed from the crossed and selfed plants was harvested when fully mature. Unfortunately, a failure in the function of the growth chamber killed most of the plants crossed with R-500 before the seed could be harvested.

Twenty plants of the progeny of each individual cross and 5 S1 plants of each pollen parent used in the crosses were grown in the greenhouse during the summer of 1985 in Jokioinen, Finland. The design was completely randomized. Most of the flowering occurred in late June and early July. The average daily maximum temperature in the greenhouse during the flowering season was 30°C and varied between 25° and 35°C. The photoperiod was over 18 h over most of the flowering period.

The level of sterility of plants in each cross was observed using the method described above. The male parents from the cultivars Nopsa, R-500, and TL-15 that had produced completely male sterile progenies when crossed with male sterile TL-15 (pol) were identified and their S1 progenies were used to pollinate the male sterile progenies of the respective cultivars.

The next generation was grown and observed during the spring and early summer of 1986 in the greenhouse in Jokioinen. Flowering occurred from the end of April to the beginning of June. The daily maximum temperatures were slightly over 30°C, and the highest temperature was 39°C. The plants required frequent watering during the periods of high temperature but no damage to the plants was observed. The photoperiod during the flowering season was between 16 h and 19 h.

In order to provide a check over environmental effects, a sample of progenies from the previous generation was grown together with this generation. Due to the limited amount of seed available, the number of crosses represented in each control group was small; 20 crosses of TL-15, 19 of Nopsa, and 7 of R-500.

The chi-square method was used to compare the data from the progenies from crosses with the selected S1 male parents with the data from the progenies of randomly chosen male parents grown earlier. The results from the control group over the two years were analyzed using the chi-square method. A comparison of the results of these analyses formed the basis for drawing conclusions regarding the effects of selection. The observed segregation for male fertility was compared with some expected ratios using chi-square analysis.

5.4 Results and Discussion

Varying degrees of male sterility can be expected in the progenies from crosses with male sterile plants when the pollen parents have not been selected for their ability as maintainers. If male fertility in the pol cytoplasm is restored by a dominant gene (or possibly several dominant genes), and the environment has little effect on the expression of fertility, the progeny from the next generation of crosses, with selected inbred male parents, should be completely male sterile.

If the maintenance of male sterility in the crosses with S1 is complete, the level of sterility obtained and its distribution in the progeny should give valuable information on the genetics of maintenance of male sterility and restoration of male fertility in B. campestris with pol cytoplasm.

A total of 946 plants was observed in the progeny from crosses with cultivar Nopsa, 352 of which were male sterile, 158 partially male sterile, and 436 fertile (Table 5.1). Only one of the 49 crosses

between TL-15 (pol) and Nopsa produced completely male sterile progeny. This finding suggested that the male parent lacked dominant restorer genes. Four of the crosses produced completely fertile progeny which indicated that the male parents used in these crosses were homozygous for at least one dominant restorer gene. In addition, two crosses produced only fertile and partially fertile progenies suggesting either incomplete expression of the restorer gene or some more complex system of inheritance of male fertility. One progeny segregated into male sterile and fertile plants with an observed ratio of 8 : 12, which did not significantly differ from a 1 : 1 ratio (X^2 = 0.80 N.S.), indicating that the male parent may have been heterozygous for one dominant restorer gene.

The remaining 34 progenies consisted of fertile, male sterile and partially male sterile individuals, which suggested either incomplete expression of the restorer gene or a more complex mechanism of inheritance.

Only 19 progenies were obtained from the crosses involving R-500 as the male parent because of the failure in the function of the growth chamber. No new crosses were made since it was assumed that the cultivar R-500 would be adequately represented by the remaining 19 individuals used as male parents.

All progenies obtained from the crosses with R-500 were observed in the greenhouse. A total number of 330 plants was observed in the progeny of R-500, 294 of which were male sterile, 13 partially male sterile and 23 fertile. Thirteen of the crosses produced completely male sterile progenies and one cross produced two partially male

sterile plants in addition to complete male sterile plants. One progeny consisted of 9 male sterile and 11 fertile plants which corresponds well to a 1 : 1 ratio (X^2 = 0.20 N.S.). The male parent may have been heterozygous for a single dominant restorer gene. The four remaining progenies consisted of male sterile, partially male sterile and fertile plants. The segregation in these progenies was not explained by any monogenic ratio.

A total of 41 crosses were made using individual plants of the parent TL-15. Of a total of 801 progeny plants observed, 750 were completely male sterile, 46 were partially male sterile and 5 were considered fertile. Twenty-two of these crosses produced entirely male sterile progenies and 19 produced some partially male sterile plants in addition to completely male steriles. One male fertile plant was observed in each of five crosses.

The results from these F_1 indicate that either a dominant restorer gene or several dominant restorer genes for the pol cytoplasm are present in B. campestris. The number of fertile individuals in many of the cross progenies was very small and many partially sterile individuals were observed. Therefore, the action of these genes do not explain adequately the whole of the variation in male sterility or fertility observed in this material.

The results from both generations of progenies produced for this experiment are summarized in Table 5.2. The distribution of individuals in the two fertility classes was significantly different in the two generations. Because selection for maintenance of complete male sterility was the main subject of this project, only two classes

were considered: male sterile vs. partially male sterile and fertile. In the generation produced using selected S1 plants as pollen parents the frequency of the male sterile plants was significantly higher than in the first generation. Since the results with the control group did not differ significantly between the two years, selection rather than environment increased the frequencies of male sterile plants in the progenies of each cultivar involved.

Heritable variation with respect to the maintenance of male sterility in pol cytoplasm exists in all three cultivars and this variation is expressed in the greenhouse environment. Since the cultivars Nopsa, R-500 and TL-15 differ widely in genetic background it seems possible that maintainer lines could be selected from a range of B. campestris cultivars. A diversity of sterile lines for hybrid production could be produced without extensive crossing programs.

None of the cultivars produced completely male sterile progenies although the percentages of male sterile plants in the progenies of crosses with S1 plants selected from R-500 and TL-15 were high (98,9 % and 99.3 % respectively). In rape (Shiga 1976) and barley (Ahokas 1979) observed that CMS plants tended to produce more pollen in the greenhouse than in the field. This may also be the situation with B. campestris (pol). At least two cycles of selection combined with inbreeding will be needed to develop strains that maintain an adequate level of sterility for the production of uniform hybrids.

The main purpose of this project was to demonstrate the feasibility of selection for maintenance of sterility in pol cytoplasm. However, the segregation for male sterility and male fertility in the progenies from crosses between completely male sterile B. campestris (pol) and selected inbred male parents provides some information on the inheritance of fertility restoration in pol cytoplasm.

Three of the crosses with S1 from Nopsa produced progeny that segregated into the classes male sterile, partially male sterile and male fertile plants. Fifteen male sterile or partially male sterile and five male fertile plants were found in progenies of each cross which corresponds to the 3 : 1 ratio expected assuming a recessive monogenic inheritance (X^2 = 0.00 N.S.). The progeny of four crosses had completely male sterile and partially male sterile progeny. The number of partially male sterile plants did not fit any monogenic segregation ratio.

Five of the crosses with S1 of R-500 and two of the crosses with S1 of TL-15 produced some partially male sterile plants. Observed segregation could not be readily explained by any monogenic pattern of inheritance.

Ahokas (1979, 1980) studied cytoplasmic male sterility in barley. He concluded that among the partial restorers, there may be semidominant single gene restorers. In some instances, if not all, partial restoration depended on more than one allele. Partial restoration was also found to be influenced by the environment.

In the present study, the results point to the existence of a single partial restorer gene which in the homozygous condition is able to restore complete or almost complete male fertility. As indicated by the low numbers of partially sterile plants in the progenies, the expression of the gene in the heterozygotes seems to be affected either by the genetic background or environment, or both. Rigorous selection is needed in B. campestris (pol) to develop lines that maintain stable male sterility.

Crosses between sibling plants were avoided in this study because inbreeding might have confounded the effect of the maintainer or restorer genes. If these conclusions are correct, inbreeding should be practised from the beginning of the selection program. The families having partially dominant restorer genes conditioning incomplete restoration could then be detected. There is no reason on the basis of this study to avoid inbreeding in the hybrid breeding of B. campestris.

The present results suggest that the initial selection for maintenance of male sterility in B. campestris (pol) can be done in the greenhouse. However, the final selections should be done in the field, in the actual environment where hybrid production is planned.

TABLE 5.1.

Fertility level of progenies from crosses of male sterile *B. campestris* x pollen parents either selected or unselected for their potential as maintainers

Male parent	Sterility or Fertility of the Progeny Groups			
	Sterile	Intermediate or segregating	Fertile	Total no. of crosses
Nopsa, original	1	44	4	49
selected	2	7	0	9
R-500, original	13	6	0	19
selected	48	5	0	53
TL-15, original	22	19	0	41
selected	47	3	0	50

TABLE 5.2

Effect of selection and environment on male fertility in progenies of crosses involving male sterile B. campestris (pol), individuals from three B. campestris cultivars and inbred progenies of plants selected for their ability to maintain male sterility

Effect of selection and environment

Male parent	Year	Progeny: Sterile	Progeny: Part. sterile/ fertile	Progeny: Total	Contingency X^2 (years x sterility)
Nopsa, original	1985	352	594	946	
selected	1986	127	45	172	78.31***
R-500, original	1985	294	36	330	
selected	1986	1033	12	1045	68.05***
TL-15, original	1985	750	51	801	
selected	1986	956	7	963	41.99***

Effect of environment on the control group*

Male parent	Year	Progeny: Sterile	Progeny: Part. sterile/ fertile	Progeny: Total	Contingency X^2 (years x sterility)
Nopsa, original	1985	140	240	380	
original	1986	155	225	380	1.09 N.S.
R-500, original	1985	117	21	138	
original	1986	119	21	140	0.01 N.S.
TL-15, original	1985	382	13	395	
original	1986	387	11	398	0.05 N.S.

* Consists of the same group of progenies from original crosses grown in both years

VI CHAPTER

MALE FERTILITY RESTORATION IN *BRASSICA CAMPESTRIS* L. WITH *POL* CYTOPLASM

6.1 Abstract

Twenty strains and cultivars of oilseed turnip-rape (*Brassica campestris* L.) from five countries were tested for their ability to restore fertility to male sterile *B. campestris* (*pol*). The highest frequencies of male fertile plants were found in the progenies of the Swedish cultivars Ante and Sv 03201. The Chinese cultivar 'Golden', as well as Indian cultivars 'BSH-1' and 'Pusa Kalyani', produced only sterile progeny.

The inheritance of the restoration of full fertility was studied in progenies of test crosses and self-pollinated progenies of fertile individuals of the test cross progenies. Two genes were found to independently restore fertility and at least one of which was completely dominant to the maintainer gene. Therefore, a minimum of two recessive alleles are needed to maintain complete male sterility in *pol* cytoplasm. The genetic background may have a significant effect in modifying the level of fertility or sterility caused by the major genes.

6.2 Introduction

Several recent reports have indicated significant heterosis for seed yield in oilseed rape (Hutcheson et al. 1981, Lefort-Buson and Dattee 1982, Sernyk and Stefansson 1982, Grant and Beversdorf 1985). This has stimulated considerable interest in the development of hybrid cultivars of various types of this crop, including turnip-rape, which is widely grown especially in Canada, India and Northern Europe. Commercial utilization of the heterosis will, however, depend on the development of a suitable pollination control mechanism. Cytoplasmic male sterility (CMS) has been used in many crops and probably offers the best prospect for use in Brassica oil crops as well.

Ohkawa (1985) proposed that nap cytoplasm, originally found in B. napus, could be used for F_1 seed production in B. campestris crops. Both maintainer and restorer genes for nap cytoplasm are found in this species (Shiga et al. 1977, Ohkawa and Shiga 1978, Ohkawa 1985). The inheritance of the sterility in nap cytoplasm is complex. Up to five dominant genes are involved in restoration of fertility in B. napus (Shiga 1976, Shiga et al. 1983, Sernyk 1982).

The temperature sensitivity of the nap male sterility mitigates against its use in commercial hybrid cultivars. Ohkawa and Shiga (1981) observed pollen development in male sterile Chinese cabbage (B. campestris ssp. pekinensis) at a temperature of 20°C. Fan and Stefansson (1986) observed that in oilseed B. napus pollen developed in male sterile plants when the day temperature was 26°C. The male sterile plants also produce pollen toward the end of their flowering

period (Shiga and Baba 1973, Ohkawa 1981).

Fu (1981) reported that cytoplasmically inherited male sterility had been discovered in B. napus variety 'Bo Li Ma' from Poland. The name Polima has also been used for this cultivar and the cytoplasm was designated pol cytoplasm by Fan (1985). Fan also observed that the stamen ontogeny is similar in sterile B. napus plants with pol and nap cytoplasms. Fan and Stefansson (1986) found that pol cytoplasm is more stable at high temperatures than nap cytoplasm. The strains that restore fertility in nap cytoplasm usually do not restore fertility in pol cytoplasm. Thus the two cytoplasms are distinct (Fan et al. 1986).

It seems to be generally agreed among plant breeders that of the male sterility inducing cytoplasms available in Brassica crops to day, pol cytoplasm is the most likely option for commercial hybrid production in the immediate future. Nonetheless, very little published information is available on the maintenance of sterility or restoration of fertility in pol cytoplasm, especially in B. campestris.

The purpose of this work was to investigate the ability of different strains of spring oilseed B. campestris to act as either maintainers or restorers for pol cytoplasm and to study the inheritance of full male fertility in B. campestris (pol). This also has the benefit of selecting stable inbred restorer lines for the pol cytoplasm in B. campestris.

6.3 Materials and Methods

Male sterility of the *B. campestris* (pol), used as the female parent in the initial crosses of this study, was maintained by crossing the pol source *B. napus* with individuals of the Indian cultivar TL-15 for five generations after a cross with an unidentified strain of *B. campestris*.

All plants for this study were grown in 10 cm flowerpots filled with a commercial peatmoss-clay-sand mixture. The plants were watered once or twice a day and a horticultural fertilizer was applied twice. The growth of the plants was normal and few plants were lost. No serious problems were caused by plant diseases or pests.

Three experiments were conducted to evaluate restoration of male fertility in *B. campestris* (pol).

1. Twenty different strains of spring turnip rape (*B. campestris*) from five countries were used as male parents in crosses with male sterile TL-15 (pol) BC_5. All plants used for crosses were grown in the greenhouse during the spring of 1985 in Jokioinen, Finland. Daily maximum temperatures were usually slightly over 30°C during the flowering season, and even on overcast days temperatures rose above 25°C. The night temperatures were 16° - 18°C.

The anther development and pollen shed of the plants was observed twice, first when flowering started and then again two days later. Only completely male sterile *B. campestris* (pol) individuals were used as female parents. Of the 559 TL-15 (pol) BC_5 plants grown, 519 were completely sterile with no observable pollen shed. The rest of the plants exhibited some evidence of pollen development although the anthers usually were non-dehiscent.

During the summer of 1985, 10 F_1 individuals of each cross were grown in the greenhouse. A completely randomized design was used. On the basis of visual observation the flowers were ranked into three classes with respect to anther development and pollen shed: 1. completely male sterile with small, white, conical anthers and no visible pollen shed; 2. partially male sterile, anthers poorly developed, but showing some pollen shed; and 3. male fertile, anthers normal or nearly normal, an abundance of pollen. The ranking of the plants was always made on the basis of the highest class of fertility observed on a given plant over the two observations.

The observations were made from the end of August to the middle of September. The natural daylength was approximately 14.5 h in the beginning of the period and 13.5 h at the end of it, but the photoperiod was extended to 18 h with artificial illumination provided by gro-lux (wide spectrum) fluorescent tubes. Daily maximum temperatures during the flowering period were between 25° and 30°C. Night temperatures varied very little: from 11.5 to 12.0°C.

2. In order to gather information about the inheritance of male fertility in pol cytoplasm and to confirm the fertility restoration observed in F_1, test crosses were made between plants classified male sterile and male fertile.

Progenies from 110 test crosses were grown in the greenhouse during the spring of 1986 in Jokioinen, Finland. Twenty plants were grown from each progeny and the design was completly randomized. Some extra plants of each progeny were grown and were used as replacements if the original plant was lost before the observations were made.

The plants were ranked for anther development and pollen shed as described above. The observation period lasted from the middle of March to the first week of April, although some individual plants were observed as late as April 15th. The natural photoperiod in the beginning of the observation period was 12.5 h and at the end 14.0 h, and artificial illumination was used as described above. Daily maximum temperatures varied from 20° to 25°C and night temperatures from 11° to 12°C.

3. Completely fertile individuals (class 3) of 12 test cross progenies were self-pollinated using the bud pollination method (Pearson 1932). Open flowers and the buds just about to open were removed, as well as the small buds on the top of the inflorescence, leaving five to ten buds to each plant. The buds were then opened and pollen of the newly opened flowers of the same plants was applied to the stigma directly from the anthers. Special care was taken to avoid cross pollination. With few exceptions, the seed sets of the self-pollinated plants were very good.

A maximum of one hundred plants from each progeny of the self-pollinations were grown in the summer of 1986 in the greenhouse in Jokioinen, Finland. The design of the experiment was again completely randomized. Twenty individuals from each test cross progeny from which selfed progenies were derived were also grown. Flowering occurred from the end of June until the middle of August. The photoperiod at the beginning of the flowering period was almost 19 hours and approximately 16.5 when the last observations were made. No artificial lights were used. Day temperatures were generally around

30°C, varying between 25° and 35°C. Night temperatures varied from 9° to 15°C. The male sterility of the plants was observed and ranking was made as before.

Statistical analyses on the data were done using the chi-square method.

6.4 Results and Discussion

6.4.1 Restorers of male fertility in the pol cytoplasm in B. campestris

Progenies were obtained from 418 crosses involving male sterile B. campestris (pol) and twenty different strains and cultivars.

Substantial variation was observed in male sterility and fertility in the progenies of different strains and cultivars (Table 6.1). The highest frequencies of fertile plants were found in the progenies from two Swedish cultivars, Ante and Sv03201, 42.9 % and 43.9 %, respectively. No completely fertile plants were found in the progenies of the Chinese cultivar 'Golden' or the Indian yellow sarson cultivars 'Pusa Kalyani' and 'IARI. These three cultivars were self fertile and homogeneous in appearance. The number of fertile plants exceeded the number of completely male sterile ones in the progeny of one cultivar only, Sv03201. Two cultivars, Ante and 70-9558, produced equal numbers of fertile and sterile offspring.

The highest frequencies of completely male sterile plants were observed in the progenies of cultivars 'Golden' (97.0 %), 'IARI' (95.2 %) and 'Pusa Kalyani' (94.9 %) as well as in the progeny of a toria type cultivar 'ITSA' from India (94.8 %). The lowest frequency

of sterile plants, 32.1 %, was found in the progeny of the Swedish strain Sv03201.

Partially male sterile plants were not predominant in any of the progenies. The highest frequencies of these were produced by cultivars Nopsa and Tobin, 32.1 % and 33.2 %, respectively. The lowest numbers of partially sterile individuals occurred in the progenies of those cultivars that did not produce any fully fertile offspring. Only six individuals, comprising 3.0 % of the total, in the progeny of the Chinese cultivar 'Golden' could not be ranked as completely male sterile as the anthers displayed some signs of pollen shed.

Although the strains and cultivars studied are not sufficiently homogeneous for use as maintainers or restorers in hybrid production, the segregation for male sterility and fertility in the crosses indicates that both maintainers and restorers for pol cytoplasm could be selected from a number of turnip-rape cultivars. Maintenance was more common than restoration. This proponderance of maintainers favors the use of the pol cytoplasm in the hybrid breeding in B. campestris.

TABLE 6.1

Male sterility and male fertility in the F_1 generation of crosses involving male sterile *B. campestris* (pol) and strains and cultivars of spring turnip-rape (*B. campestris*)

Strain or Cultivar	Country of Origin	No. of Crosses	No.of Plants Observ.	Male Fertility of Plants		
				Part. Sterile	Sterile	Fertile
Bird Rape	Canada	33	329	259	52	18
Span	Canada	15	150	110	29	11
Tobin	Canada	25	247	140	82	25
Torch	Canada	22	212	173	35	4
Golden	China	20	197	191	6	0
Qingyou Wuhao	China	13	130	89	29	12
Nopsa	Finland	27	265	117	85	63
BSH-1	India	28	277	237	13	27
IARI	India	13	125	119	6	0
ITSA	India	22	213	202	7	4
Pusa Kalyani	India	24	234	222	12	0
Toria-7	India	20	199	173	13	13
Ante	Sweden	20	191	82	27	82
Emma	Sweden	19	186	100	42	44
Sv 03201	Sweden	23	221	72	52	97
Sv 68-420	Sweden	18	177	136	25	16
Sv 7510087	Sweden	21	209	105	49	55
Torpe	Sweden	21	199	135	40	24
Tyko	Sweden	19	187	118	57	12
70-9558	Sweden	15	148	58	32	58
Total		418	4096	2838	693	565

6.4.2 Test Crosses Involving Male Sterile B. campestris (pol) and Heterozygous Restorers

A total of 110 test cross progenies were included in this study. Twenty plants of each were grown and observed. In 51 of the progenies some partially male sterile plants were found while the rest of the crosses produced only male fertile and completely male sterile offspring. The distribution of fertility in the material is shown in Figure 6.1.

One of the progenies observed was entirely male sterile. The maximum number of fertile plants per progeny was 19, all of which were observed in one progeny. The highest frequencies of progenies occurred in the classes with 10, 11 and 12 fertile plants per progeny. There were 15 progenies in each of these classes. The occurrence of one entirely male sterile cross among the test cross progenies may indicate instability in the expression of the fertility restoration as the fertility observed in the heterozygous male parent was not expressed in the progeny.

Chi-square tests were not used to evaluate this material because the small sample size would seriously limit the validity of the test at both ends of the distribution, where the results are of most interest. However, samples of twenty plants from twelve of the test cross progenies were grown later in the same year together with the selfed progenies derived from them. The combined results for these twelve progenies are presented in Table 6.2. Since the frequencies of partially male sterile plants were very low, classes 1 (male sterile) and 2 (partially male sterile) were combined for statistical analysis.

In seven of these groups (2, 3, 4, 6, 9, 10 and 11) the segregation ratios agreed well with the ratios expected on the basis of a dominant monogenic inheritance. In four groups (1, 7, 8 and 10) the ratios did not significantly differ from the ratio of 3 fertiles : 1 sterile expected on the basis of two dominant genes, each acting independently. However, in two of these cases (1 and 8) the ratio did not significantly differ from 1 : 7 ratio, which should result if the fertile male parent is a heterozygote for three unlinked dominant restorer genes acting independently. In two cases (5 and 12) it was difficult to draw a definite conclusion, since the results from the two experiments were very different.

The restorer genes in test crosses 1 and 8 were derived from the Swedish cultivar Ante, which seems to have at least two dominant restorer genes for pol cytoplasm. The results from the two crosses, 2 and 7, involving Torch differed, indicating 1 or 2 completely dominant restorer genes in this cultivar. The other cultivars used as sources of fertility restoration in these test crosses were: Itsa (test cross 6), Nopsa (11), Sv 3201 (4, 5 and 10), Sv 7510087 (12), Quinqyou Wuhao (9) and Tobin (3).

These results suggest that the restoration of fertility is controlled by a single dominant gene in some crosses, and by at least two dominant genes in other crosses.

TABLE 6.2

Segregation of male sterility and fertility in the test cross progenies of B. campestris (pol)

	Observed segregation		X^2 for expected ratios		
	Sterile or partially sterile	Fertile	1 : 1	1 : 3	1 : 7
Test cross 1.	6	34	19.60***	2.13	0.23
heterogeneity			0.40	0.54	0.91
Test cross 2.	21	19	0.10	16.13***	58.51***
heterogeneity			0.10	0.14	0.23
Test cross 3.	26	17	3.60	34.13***	100.80***
heterogeneity			1.60	2.14	3.66
Test cross 4.	20	20	0.00	13.33***	51.43***
heterogeneity			0.40	0.54	0.91
Test cross 5.	15	25	2.50	3.33	22.86***
heterogeneity			4.90*	6.54*	11.20***
Test cross 6.	21	19	0.10	16.14***	58.51***
heterogeneity			0.90	1.21	2.06
Test cross 7.	10	30	10.00**	0.00	5.71*
heterogeneity			0.40	0.54	0.92
Test cross 8.	6	34	19.60***	2.13	0.23
heterogeneity			1.60	2.14	3.66
Test cross 9.	17	23	0.90	6.53*	32.91***
heterogeneity			2.50	3.34	5.72*
Test cross 10.	15	25	2.50	3.33	22.86***
heterogeneity			0.90	1.21	2.05
Test cross 11.	18	22	0.40	8.53**	38.63***
heterogeneity			0.40	0.54	0.91
Test cross 12.	12	28	6.40*	0.53	11.20***
heterogeneity			3.60	4.81*	8.22**

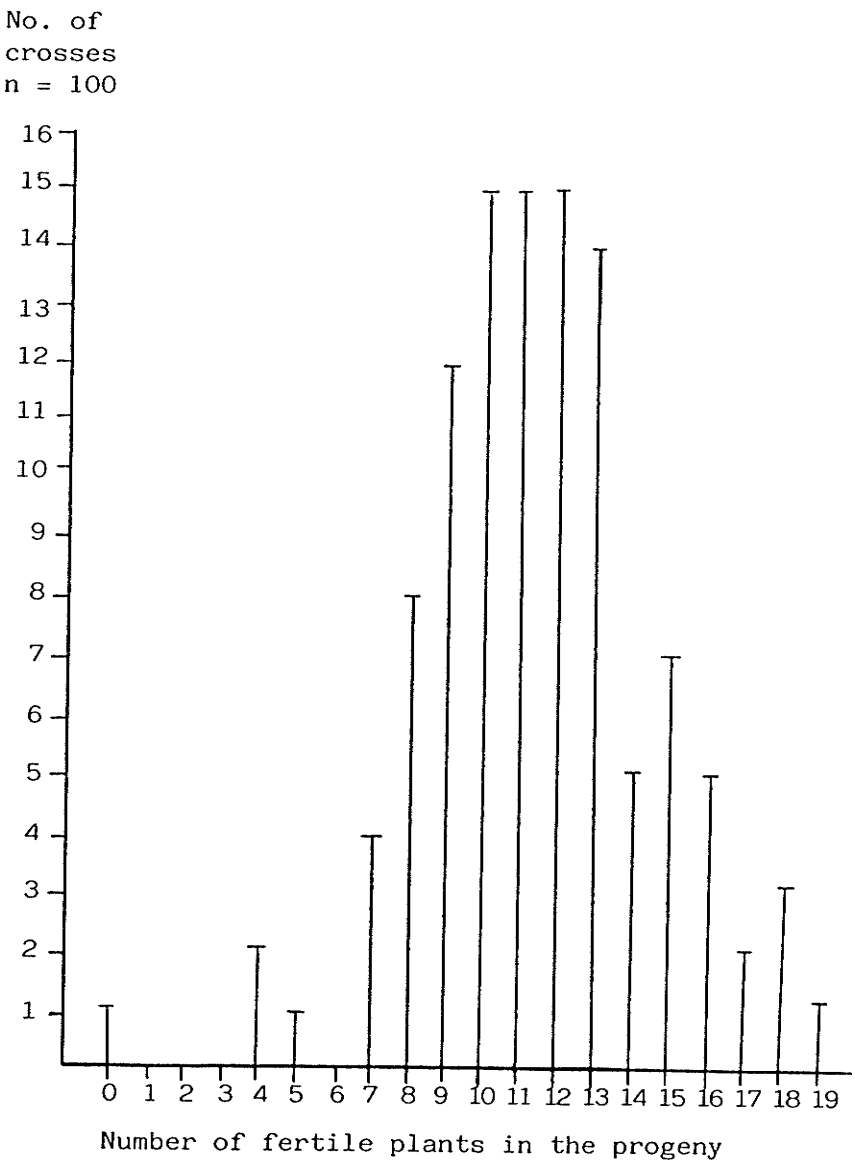


Figure 6.1: Distribution of male fertility among 110 test cross progenies of B. campestris (pol).

6.4.3 Progenies of Self-Pollinated Male Fertile Plants from the Test Crosses

Forty two progenies derived from the self-pollination of 12 test cross progenies were grown and observed. The maximum number of plants per progeny was one hundred. In two of the progenies no partially sterile plants could be observed, while all others consisted of male sterile, partially male sterile and male fertile plants. For statistical analysis classes 1 (completely male sterile) and 2 (partially male sterile) were combined.

On the basis of the results from the test cross progenies both monogenic and digenic segregation ratios were expected. The frequency of families showing digenic segregation ratios was expected to be low, since with independently segregating restorer genes, only one out of three heterozygous fertile plants would carry both genes. Furthermore, it was expected that many of the 12 progeny groups, each derived from different test cross progenies, would show homogeneously monogenic segregation ratios while those groups in which digenic ratios occurred would be heterogeneous.

The expected ratios against which the observed segregation ratios were tested were ((sterile plants + partially sterile plants) : fertile plants) and the corresponding mechanisms of inheritance are briefly listed below:

3 : 1, only one recessive or partially dominant restorer gene present in the progeny, assuming that in the case of partial dominance the heterozygotes are partially sterile;

9 : 7, two recessive or partially dominant restorer genes, either of which is able to restore male fertility in homozygotes;

7 : 9, two complementary dominant restorer genes, full fertility is restored only if both are present in the same plant, otherwise the plants are partially or completely sterile;

1 : 3, one completely dominant restorer gene present;

3 : 13, one dominant and one recessive or partially dominant restorer gene present in the progeny;

1 : 15, two dominant restorer genes present, either of which alone is sufficient to restore full fertility.

The Chi-square analysis of the progeny groups derived from 12 test cross progenies can be seen in Tables 6.5. Six progeny groups (3, 4, 5, 6, 7 and 10) exhibit segregation ratios that do not differ significantly from the expected 1 : 3 ratio. The ratios obtained from selfed progenies agree with the ones from the respective test crosses. One of the groups of selfed progenies showing homogeneous segregation for one gene was derived from a test cross progeny that was hypothesized to carry two dominant genes for fertility restoration, but since only two plants of that progeny (test cross 7) were self-pollinated these results could be due to sampling error.

In one group (11) the observed overall segregation does not differ significantly from a ratio of 1 : 3 expected if one dominant restorer gene is present. However, significant heterogeneity exists which indicates that some individual progenies within the group deviate from the expected ratio (1 : 3). The four individual selfings in this group show different digenic segregation ratios for both

dominant and recessive genes. It is possible that in addition to one dominant restorer gene one or more recessive restorer genes also condition male fertility in this group.

Two groups, 2 and 8, differ significantly from the expected 1 : 3 ratio, without showing significant heterogeneity. In group 2 one selfed progeny (progeny 2.3, table 6.4) segregates in a ratio approaching 7 : 9 while three are segregating in 1 : 3 ratio. In group 8 all five selfed progenies fit a ratio of 3: 13 (progenies 8.1 - 8.5, Table 6.4), and in two cases the ratio does not differ significantly from 1 : 3 either. Since the results in both groups are difficult to explain on the basis of segregation in either test crosses or selfed progenies, it seems possible that the observed segregation ratios in these groups are biased by effects of environment or genetic background.

The three groups (1, 9 and 12) that show overall segregation ratios significantly deviating from 1 : 3 (Table 6.5), are heterogeneous.

In group 1 (Table 6.4 progenies 1.1 - 1.4) the segregation ratios indicate that in addition to a dominant restorer gene a recessive gene was also present in the test cross. Since the proportion of partially sterile plants in the selfed progenies is disproportionally high, the restorer may have some effect on the fertility in heterozygotes, and thus is acting as a partially dominant rather than a completely recessive gene. The results from the test crosses indicated the presence of two dominant restorer genes. Although this difference in the results from the test crosses

and selfings could be due to sampling error, it is also possible that the gene expressing partial dominance could be completely dominant in a different environment or genetic back-ground and thus give rise to different segregation ratios. One progeny (progeny 1.3, Table 6.4) in this group gives a segregation ratio of 7: 9 indicating complementary gene action. Since the results of either the test cross or the remaining selfed progenies in group 1 do not support complementary gene action in the inheritance of male fertility, this result is difficult to explain and may be due to environmental effect on male fertility.

In group 9 the results from the individual selfed progenies (progenies 9.1. - 9.3, Table 6.4) suggest the presence of one dominant and two recessive or partially dominant genes in the respective test cross progeny. The results of the test cross 9 also indicated that one dominant restorer gene was present.

In group 12 the results from individual selfed progenies (Table 6.4, progenies 12.1 - 12.4) indicate that at least two genes affecting male fertility are present and that these genes may also be complementary. The number of restorer genes present in test cross progeny 12 could not be defined because of the significant heterogenity between the two experiments in which the test cross progeny was grown. It is possible that in this group the restoration of male fertility is complex and significantly affected by environment.

In all individual selfed progenies, the segregation for complete male fertility could be explained by the presence of major genes at

one or two loci with varying degrees of dominance. This variation in the degree of dominance could be due to the genetic background, or environment. It is also possible that one or both of the loci have several alleles with different effects on male fertility in the pol cytoplasm.

The frequencies of partially male sterile plants do not fit well into any expected pattern of monogenic or digenic inheritance. This is especially evident in progenies where the restoration of full fertility seems to be conditioned by one dominant gene, since in most of them there are also a variable number of partially fertile plants whose presence can not be explained by the effect of the major gene. It is likely that in some instances male sterility also depends on polygenic effects that modify the effect of the major restorer genes.

Although the variation in the expression of partial sterility makes it somewhat difficult to interpret these results, it seems probable that at least two major recessive alleles in a suitable genetic background are needed to maintain complete male sterility.

A cytoplasmic-genic male sterility in which sterility is controlled by two recessive alleles was also found in sugar beet by Owen (1945). He also observed that the expression of partial male sterility in this system was very unstable.

CMS systems with a completely dominant restorer or restorers seem to be far more common than partially dominant or recessive systems. This might be explained by the history of the material used in the present study. Since one of the purposes of this study was to develop inbred lines that would restore full fertility to crosses

with sterile B. campestris (pol), completely fertile heterozygous plants were selected for both test crosses and for the derivation of selfed progenies. Such a procedure was designed to increase the frequency of dominant restorer genes over the partially dominant genes. Where the effects of the restorer genes are modified by polygenes, the procedure also favours a type of genetic background in which complete dominance in fertility restoration is expressed.

It is likely that the effects of both restorer and maintainer genes are modified by environment as well as by the genetic background. Instability in the expression of male sterility and fertility may be a characteristic feature of certain genotypes rather than of the cytoplasm in general. With appropriate methods of selection, both stable fertility and sterility should be attainable. Thus the prospects for the use of pol cytoplasm in hybrid breeding of B. campestris are promising.

TABLE 6.4

Segregation for male fertility and male sterility in the F_2 generation derived from self pollinated *B. campestris* (pol) plants heterozygous for fertility restoration

	Observed segreg.			X^2 values for expected segregation ratios ((sterile + partially sterile) : fertile)					
Prog.	Ster.	Part. Ster.	Fert.	3:1	9:7	7:9	1:3	3:13	1:15
1.1	48	31	19	1.65	23.62***	54.11***	161.65***	245.37***	924.87***
1.2	53	25	22	0.48	19.21***	47.67***	149.81***	229.67***	878.60***
1.3	10	29	61	69.12***	12.07***	0.92	10.45***	26.77***	181.20***
1.4	4	7	81	195.01***	73.25***	37.79***	8.35**	2.81	5.11*
2.1	26	4	60	83.33***	19.18***	3.97*	3.33	12.48***	112.67***
2.2	17	10	71	117.67***	32.75***	10.45**	0.34	4.94*	75.89***
2.3	26	15	59	59.67***	9.45**	0.31	13.65***	32.33***	206.09***
2.4	24	3	73	122.88***	34.72***	11.40***	0.21	4.42*	73.48***
3.1	15	1	36	54.26***	13.70***	3.56	0.92	4.89*	53.35***
3.2	9	7	72	151.52***	51.75***	23.38***	2.18	0.02	21.38***
3.3	22	1	77	144.21***	44.86***	17.50***	0.21	1.17	47.88***
4.1	26	5	69	103.25***	25.87***	6.61*	1.92	9.78**	104.54***
4.2	13	5	73	147.99***	49.11***	21.25***	1.32	0.06	28.43***
4.3	19	10	71	112.85***	30.13***	8.84**	0.85	6.84**	88.33***
4.4	24	8	67	96.16***	22.99***	5.25*	2.83	11.89***	114.86***
5.1	23	5	72	117.81***	32.38***	10.08**	0.48	5.57*	80.74**
5.2	18	7	75	133.33***	39.63***	14.29***	0.00	2.53	50.00***
5.3	16	2	82	173.28***	59.37***	26.94***	2.61	0.04	23.56***
5.4	19	4	76	141.50***	43.79***	16.94***	0.16	12.28	48.73***

Prog.	Observed segreg. Ster.	Part. Ster.	Fert.	X^2 values for expected segregation ratios ((sterile + partially sterile) : fertile) 3:1	9:7	7:9	1:3	3:13	1:15
6.1	8	3	49	102.76***	35.00***	15.75***	1.42	0.01	14.95***
6.2	18	2	57	98.71***	28.64***	9.89**	0.04	2.61	51.12***
7.1	17	0	83	179.41***	62.52***	29.08***	3.41	0.21	19.72***
7.2	17	3	84	144.70***	57.84***	19.29***	0.70	0.38	36.22***
8.1	12	4	82	179.93***	63.39***	29.95***	3.93*	0.39	16.98***
8.2	16	1	81	173.73***	60.19***	27.76***	3.06	0.13	20.60***
8.3	10	2	87	208.76***	78.24***	40.24***	8.76**	2.88	5.82*
8.4	17	1	69	136.86***	55.64***	18.80***	0.86	0.21	30.96***
8.5	16	4	80	161.33***	53.33***	22.92***	1.33	0.10	32.27***
9.1	3	10	86	202.10***	74.70***	37.71***	7.44**	2.07	8.00**
9.2	28	38	29	1.55	6.75**	25.54***	100.21***	159.88***	648.08***
9.3	28	34	36	7.20***	1.96	15.17***	76.53***	127.01***	543.70***
10.1	8	15	76	141.50***	43.79***	16.94***	0.16	1.28	48.73***
10.2	24	4	72	117.81***	32.38***	10.08	0.48	5.57*	80.74***
10.3	21	4	66	109.63***	30.58***	9.80**	0.30	4.50*	69.95***
11.1	8	12	31	34.83***	6.00*	0.43	5.50*	13.95***	94.59***
11.2	2	1	37	97.20***	38.58***	21.36***	6.53*	3.33	0.11
11.3	16	0	81	177.08***	29.28***	29.28***	3.74	0.33	17.38***
11.4	21	22	54	48.66***	5.59*	0.01	19.33***	41.47***	240.06***
12.1	28	10	61	70.79***	12.82***	1.16	9.46**	24.91***	174.47***
12.2	17	3	29	27.00***	4.73*	0.02	8.33**	18.60***	112.07***
12.3	47	11	40	13.07***	0.34	9.49**	61.07***	104.77***	468.64***
12.4	36	4	42	30.07***	1.85	0.84	24.73***	48.33***	253.14***

TABLE 6.5

Segregation for male fertility and male sterility in the F_2 progenies derived by self pollination from progenies of test crosses between male sterile and male fertile B. campestris with the pol cytoplasm. The frequencies of sterile and partially sterile plants were combined for X^2 analysis

Test cross	No. of Selfings	Observed segregation			X^2 value for expected ratio 1 : 3	Heterogeneity X^2
		Ster.	Part. Ster.	Fert.		
1	4	115	92	183	163.97***	166.29***
2	4	93	32	263	10.78**	6.75
3	3	46	9	185	0.56	2.75
4	4	82	28	280	2.14	4.78
5	4	76	18	305	0.44	2.81
6	2	26	5	106	0.41	1.05
7	2	34	3	157	3.64	0.47
8	5	71	12	399	15.56***	2.38
9	3	59	82	151	84.46***	99.72***
10	3	53	23	214	0.23	0.71
11	4	47	35	203	2.16	32.94***
12	4	128	28	328	91.46***	12.13**

VII CHAPTER

INFLUENCE OF GROWTH TEMPERATURE AND SENESCENCE ON MALE STERILITY IN *BRASSICA CAMPESTRIS* L. WITH *POL* CYTOPLASM

7.1 Abstract

The effect of temperature and senescence on male sterility in *B. campestris* (*pol*) was investigated. The day/night temperature regimes used were 14/6°, 22/14°, and 30/22°C. The sterility of the anthers was observed three times: at the beginning of flowering, two days later, and at the end of flowering of the main raceme. A decrease in the number of male sterile plants was observed at low temperatures and toward the end of flowering, but no complete reversion to male fertility was ever found. The results indicate that male sterility associated with the *pol* cytoplasm is sufficiently stable to be used for breeding hybrid cultivars of *B. campestris*.

7.2 Introduction

Since the discovery of cytoplasmic male sterility in the *Cruciferae* by Ogura (1968), there has been considerable interest in developing CMS systems for the hybrid breeding of rapeseed (*Brassica* sp.). Several systems that induce some degree of male sterility in *Brassica* crops are known today (Erickson et al. 1986).

The sterility inducing effect of pol cytoplasm was first reported by Fu (1981). The pol CMS has some favorable features and is being evaluated at several institutions for use in hybrid production.

Several reports on different crops indicate that temperature is one of the main environmental influences on expression of male sterility (Meyer 1969, Marrewik 1969, Estrada and Mutschler 1984). Shiga (1973) and Ohkawa and Shiga (1981) reported that male sterile rapeseed with nap cytoplasm may produce functional pollen in the late stages of flowering, especially if exposed to high temperatures. This observation was confirmed by Fan and Stefansson (1986), who also found that the pol system is affected in the same way. The temperatures required to break down the male sterility, however, were much higher than with the nap cytoplasm. The effect of high temperatures on the male sterility of B. napus with the pol cytoplasm was also reported by Fu (1981). According to his observations some plants exhibited increased fertility also in low temperatures.

The purpose of the present work was to determine if effects similar to those described for B. napus could be found in B. campestris and to gather information on the conditions in which selection should be done for the maintenance of sterility and restoration of fertility.

7.3 Materials and Methods

Thirty progenies from crosses involving male sterile B. campestris (pol) and individual plants of cultivars Nopsa, R-500, TL-15 and Tobin were subjected to three temperature treatments. The

parent cultivars were grown together with the hybrid progenies.

Three different temperature treatments were used and the experiment was repeated once. In each treatment the seeds were germinated for two days on filter paper in Petri-dishes after which ten individuals of each cross progeny and cultivar were transplanted to 10 cm plastic pots filled with a homogenous sand-peatmoss mixture. The pH of the soil was adjusted to 6.5 with lime. The pots were placed in a growth cabinet in a completely randomized design.

The plants were grown under an 18 h photoperiod. Light was provided by six multimetal lamps, type Osram HQI-T 1000 W/D. Photosynthetic photon flux density was measured at the level of the tops of the plants and varied from 290 to 340 $uEm^{-2}s^{-1}$.

The plants were watered twice a day. Liquid horticultural fertilizer was applied one week and two weeks after the planting. The relative humidity in the chambers varied from 70 % to 80 %. In the second experiment of the high temperature treatment the relative humidity could not be controlled properly and rose to 95 %.

Prior to all three treatments the plants were grown for two weeks after planting in a temperature regime of 22°C and 14°C by day/night, respectively. After this phase the plants in treatment I were moved to a cool regime of 14°C by day and 6°C by night. In treatment II, the temperature regime was continued at 22°C and 14°C. In treatment III the plants were subjected to higher temperatures, 30°/22°C day/night.

The development of the anthers was first studied when flowering started and again two days later. If variation existed between the

individual flowers the class of the most fully developed ones was recorded. After the second observation, the plants of treatments I and III were returned to the original temperatures (22°C by day and 14°C by night). The third observation on sterility was made when flowering of the main raceme of the plant was nearing completion, usually 12 to 14 days after the first observation.

On the basis of these observations, plants were classified into three classes: 1. completely sterile, no pollen shed; 2. partially sterile, a small amount of pollen shed, anthers visibly abnormal; and 3. completely fertile, abundant pollen shed, anthers normal or almost normal.

The differences in the distribution of male sterility or fertility between different observation times and treatments were analysed using the X^2 method.

7.4 Results and Discussion

The effects of the temperature treatments on plant development were clear. In treatments II (22°/14°C) and III (30°/22°C) the first flowers opened 24 days after planting. In treatment I (14°/6°C) the development was somewhat slower and the first plants flowered 30 days after planting. The plants grown constantly at intermediate temperatures developed well and looked normal. The plants grown at the lower temperatures (treatment I) were, however, much more vigorous with thicker stems and larger leaves. The colour of the leaves was also a darker green than that of the plants grown at the intermediate temperatures.

The plants grown at the highest temperatures(30°/22°C) developed abnormally with very weak stems and small leaves. Their growth improved after they had been returned to the 22°/14°C regime but they still appeared less vigorous than the plants of the other treatments. After the change of the temperature they also showed a much stronger tendency to branch than the plants kept at lower temperatures.

The plants in the field and especially in the greenhouse are often exposed to temperatures much higher than those used in this experiment without showing any of the symptoms observed. It is unlikely therefore that high maximum temperatures are the sole reason for the observed disturbances in growth. It was noticed that the characteristics described above were expressed very strongly in the second experiment where the relative humidity of the air was close to 95 % throughout most of the experiment.

With the exception of results at the highest temperature regime the distribution of male sterility or fertility was similar in both experiments (Table 7.1). The highest frequencies of male sterile plants in the cross progenies with pol cytoplasm were observed at the beginning of flowering in treatment III (the highest temperatures). However, most of the plants of the parental cultivars with normal cytoplasm were also male sterile, so the conditions must be considered exceptional. The results do not indicate any specific reaction of the material with the pol cytoplasm.

After the plants had been returned to lower temperatures, those with normal cytoplasm produced increasingly male fertile flowers and the plants soon gained full male fertility. In contrast, the

frequency of male sterile and partially male sterile plants with pol cytoplasm remained higher than in the other treatments.

The lowest frequencies of male sterile plants in the pol material occurred at the end of the flowering period among the plants kept at the lowest temperature regime. In all the treatments the plants developed more pollen toward the end of the flowering period.

The results of the contingency analyses can be seen in Table 7.2. In order to compare the results from the different treatments and observation times the data from both experiments were pooled.

Treatments I (14/6°C) and II (22/14°C) were compared separately from treatment III (30/22°C). It was thought that the high overall sterility observed at the beginning of the flowering period in the treatment III might not reflect any specific qualities of the male sterility associated with the pol cytoplasm.

The observation time and temperature treatment have a significant effect on the male sterility of the B. campestris plants with pol cytoplasm, as there are significant differences in the distribution of male sterility or male fertility both between the treatments and observation times. The difference is also significant when only treatments I and II are compared.

Low growth temperatures seem to increase the incidence of fertility and decrease sterility. The plants previously grown in low temperature regime expressed better anther development than the plants kept in higher temperatures even after they had been returned to the same temperature regime (22/14°C) for 10 - 12 days. One possible explanation for the carry-over of temperature effects is

based on nutritional differences. Plants grown at low temperatures were better developed and theoretically should be able to translocate more nutrients for flower development than poorly developed plants grown at the higher temperatures.

The specific fertility-improving effect of high temperatures, found by Fan and Stefansson (1986) in B. napus was not observed. These differences in the results may be due to the biological differences between the two species. During the breeding work on B. campestris (pol), thousands of male sterile plants were grown in the greenhouse, where temperature often was over 35°C, without causing any visible effect on male sterility. This fact, together with the results of the present study, indicate that the pol sterility in B. campestris is more stable than in B. napus.

In addition to its effect on male sterility, temperature treatment affected the development of the flowers in other ways. In many of the cross progenies and also in cultivar TL-15, the petals of the plants grown at the lowest temperature regime (16/6°C, treatment I) were about twice the size of the petals of plants from treatment II (22/14°C). Conversely, many of the plants grown at the highest temperatures (30/22°C, treatment III) developed very small petals (Fig. 7.1 - 7.3). The pedicels of the plants grown at low temperatures were also longer than those of the plants grown at higher temperatures. This affected the appearance of the inflorescence. These differences in the appearance of the flowers and inflorescences at different temperatures are especially interesting, because corresponding differences are often found when male sterile

and male fertile plants, grown together in the same conditions, are compared.

In all three treatments the fertility increased toward the end of the flowering period. Although in treatment III (30/22°C) this may have been mainly due to recovery from the injurious effects of high temperatures, it is likely that the improvement of the fertility at lower temperatures was caused by the physiological changes associated with senescence. These observations are in agreement with those made on plants with nap cytoplasm (Shiga and Baba 1973, Ohkawa and Shiga 1981, Ohkawa 1985).

Although both temperature treatment and the age of the plants had a significant effect on the level of sterility, some of the hybrid progenies remained completely male sterile at all temperatures and observation times. Thus, it appears that stable male sterility is attainable in B. campestris with pol cytoplasm and that the level of sterility is adequate for the development of commercial hybrid cultivars.

TABLE 7.1

Distribution of male sterility and fertility in B. campestris (pol) in different temperature treatments with three observation times

	Sterility* at the beginning of flowering			Sterility two days after the beginning of flowering			Sterility at the end of flowering		
Treatment	S	PS	F	S	PS	F	S	PS	F
14/6 °C									
Replication 1	162	28	110	150	22	128	110	44	146
Replication 2	168	37	95	153	37	110	122	60	118
Total	330	65	205	303	59	238	232	104	264
22/14°C									
Replication 1	207	35	58	192	46	62	164	60	76
Replication 2	208	41	51	199	43	58	178	51	71
Total	405	76	109	391	89	120	342	111	147
30/22°C									
Replication 1	233	65	2	232	66	2	184	61	55
Replication 2	296	4	0	294	4	2	186	79	35
Total	529	69	2	526	70	4	370	140	90

* S, completely male sterile; PS, partially male sterile; F, male fertile

TABLE 7.2

Distribution of male sterility or fertility in B. campestris (pol) in different temperature treatments with three observation times. Summary of contigency X^2 analyses

Comparison between three observation times, within treatments

Treatment	d.f.	Contingency X^2
14/6°C	4	122.712***
22/14°C	4	60.421***
30/22°C	4	684.629***

Comparison between three temperature treatments, whithin the observation times

Observation time	d.f.	Contingency X^2
1.	4	730.937***
2.	4	885.846***
3.	4	402.658***

Comparison between two temperature treatments, 14/6°C and 22/14°C, within the observation times

Observation time	d.f.	Contingency X^2
1.	2	119.719***
2.	2	168.400***
3.	2	163.844***

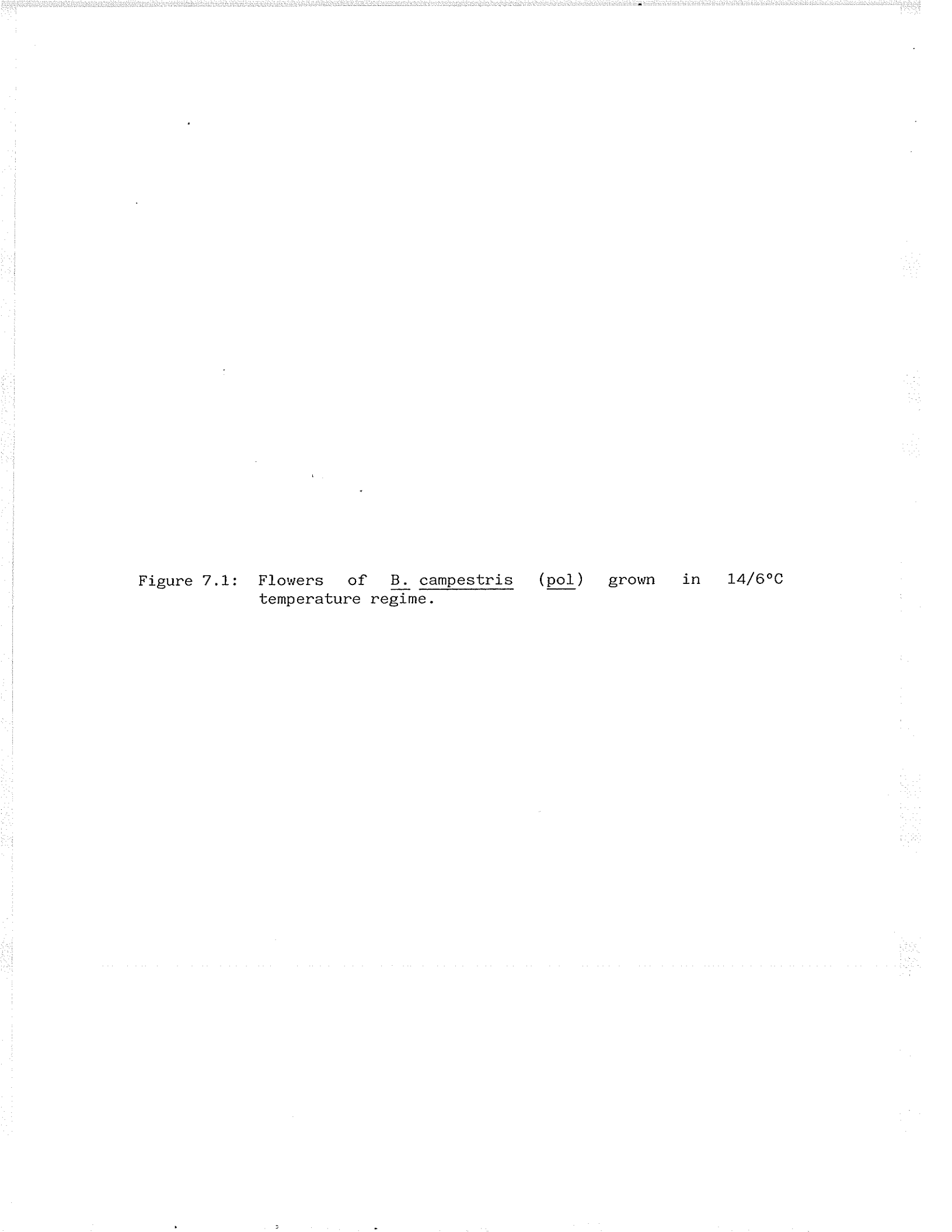


Figure 7.1: Flowers of B. campestris (pol) grown in 14/6°C temperature regime.

7.2.: Flower of B. campestris (pol) grown in 22/14°C temperature regime.

Figure 7.3: Flower of B. campestris (pol) grown in 30/22°C temperature regime.

VIII CHAPTER

GENERAL DISCUSSION AND CONCLUSIONS

Reports of significant heterosis for seed yield in Brassica campestris L., and other Brassica oilseed crops, have stimulated interest in the hybrid breeding of this species. For commercial production of hybrid cultivars a reliable and practical method for pollination control is needed. Several CMS systems that could potentially be used for the hybrid breeding of B. campestris are known, including systems conditioned by mur, nap, ogu and pol cytoplasms.

Male sterility associated with pol cytoplasm was first reported by Fu (1981) and is now widely used in the hybrid breeding of B. napus. Little information seems to be available on the effects of pol cytoplasm on B. campestris or of the mode of inheritance of the male sterility associated with this cytoplasm. Therefore, the main emphasis in this project was devoted to the solution of problems related to the use of pol cytoplasm in hybrid breeding of B. campestris.

In comparison with other male sterile cytoplasms mur, nap and ogu, pol cytoplasm was found to offer good prospects for the breeding of hybrid cultivars since both maintainers and restorers were

discovered in oilseed forms of B. campestris. In the present study no fertility restoration for the ogu cytoplasm was observed in the progenies of crosses involving 41 stains and cultivars of B. campestris. Most progenies with nap cytoplasm were completely fertile and none of the progenies was considered completely male sterile. Comparison of the results from the progenies with cytoplasms nap and pol from two locations indicated that the male sterility or fertility conditioned by nap cytoplasm was significantly affected by the environment while the progenies with pol cytoplasm were much more stable.

The form of male sterility in the mur cytoplasm used in these trials shows little promise for use in commercial hybrids. The irregular inheritance of male sterility from this source was probably caused by an extra chromosome from Diplotaxis muralis (L.) DC. reported by Fan et al. (1985).

The inheritance of the male sterility associated with pol cytoplasm was maternal and the maintainers and restorers observed are characteristic of a cytoplasmic-genic system of inheritance. The male sterile plants tended to be taller and flowered later than the male fertile plants. None of these characters found associated with pol male sterility appear to be detrimental for its use in hybrid production.

Complete male fertility in pol cytoplasm can be restored by a single dominant gene. Dominant restorer genes were observed in many cultivars, but they appeared to be especially common in some of those of Swedish origin. The allelic relationship of the restorer genes

from different sources was not studied. Recessive or partially dominant restorer genes also appeared to be present in some strains. With selection, restoration based on a single dominant gene probably could be used to develop hybrid cultivars.

A minimum of two maintainer genes appears to be needed for the maintenance of complete male sterility. Partial sterility was conditioned by a partially dominant restorer gene. The expression of partial sterility was unstable and it seems likely that it is also conditioned by polygenes and environment. Thus, rigorous selection for sterility maintenace will be needed in order to establish stable male sterility in B. campestris.

Low temperatures were observed to favor the expression of male fertility. Pollen production was observed to increase towards the end of flowering period. Since male sterile plants were observed at both low and high temperatures, environmentally stable male sterility probably is attainable with proper selection techniques. These techniques should include testing under variable field conditions in order to secure stability of the male sterility.

Although most of the cultivars restored some degree of fertility in crosses with male sterile B. campestris (pol), none of them was sufficiently homogeneous to be used as a restorer in the production of hybrid cultivars. Selection of suitable restorers from existing cultivars should, however, be possible.

The presence of self incompatibility in most strains and cultivars of different types of B. campestris is one of the difficulties in developing F_1 hybrid cultivars. The genes for self

compatibility and quality characteristics such as low erucic acid and low glucosinolate content would have to be incorporated into both maintainer and restorer lines. These lines would also have to show good combining ability and therefore probably should be derived from diverse gene pools.

Since it is possible to develop high levels of male sterility and effective restoration, the pol cytoplasm probably can be used to develop commercial hybrid cultivars in B. campestris. However, substantial efforts will be needed to transform this possibility into practical reality.

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