DEVELOPMENT AND EVALUATION OF THE DIPLOTAXIS MURALIS (L.) DC (mur) CYTOPLASMIC MALE STERILITY (CMS) SYSTEM IN SUMMER RAPE (BRASSICA NAPUS L.)

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BY

TERESIO CAESAR RIUNGU

A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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ABSTRACT

This study developed and evaluated the Diplotaxis muralis (L) D.C. cytoplasmic male sterility (CMS) system (mur) in summer oilseed rape, starting from a winter rapeseed mur CMS A-line and B-line pair obtained previously by the University of Manitoba. The objectives of the study were to; 1) search for mur CMS system maintainer genotypes in summer oilseed rape, 2) study the inheritance pattern of mur CMS maintenance and restoration in summer rape, 3) develop mur CMS A-line and B-line pairs in summer rape, 4) evaluate the effect of high temperature treatment on mur CMS A-lines in summer rape, and 5) evaluate the comparative performance of summer rape hybrids in mur and nap cytoplasms.

There were no maintainer genotypes found in any of the 101 summer oilseed rape lines and cultivars evaluated, i.e. they were all restorers and, therefore the frequency of occurrence of maintainer genotypes in summer oilseed rape is low or zero. One to three genes controlled the maintenance and restoration of male sterility for mur CMS. Cultivars differed in the number of genes they carried for maintenance and restoration. Three pairs of mur CMS A-lines and B-lines in summer rape were developed. The male sterility of the mur CMS A-lines was stable up to $30/24^0$ C. Hybrids in both mur and nap cytoplasms exhibited superior relative performance for seed yield compared to their open pollinated population parents. The mur and nap hybrids were not significantly different for days to emergence, seedling vigour, days to flowering and maturity, plant height, seed yield, total dry matter, and harvest index at any of four environments tested, but the hybrids were significantly different at all four environments for oil content. Averaged over

environments, hybrids in the mur cytoplasm performed significantly poorer than hybrids in the nap cytoplasm for seed yield, total dry matter and oil content but significantly better for protein content, suggesting some pleiotropic negative effects, (i.e. biological costs) associated with the mur cytoplasm. Nonetheless, the mur CMS system has good potential for use in hybrid summer rape cultivar development programmes.

1. INTRODUCTION

Summer rape (Brassica napus L.), a member of the Brassicaceae family is an important oil crop in cool and humid agricultural areas of the world, and the most important oilseed species grown in western Canada. Summer rape research in Canada initially focused on improvements in seed component quality (i.e. low erucic acid in the oil and low glucosinolate levels in the meal, Downey et al. 1975), producing a new commodity termed "canola" from double low summer rape cultivars. Research also focused on improvements in summer rape productivity and this has led to increased summer rape production in Canada in the last 30 years. Canada is a major producer of canola and a major exporter of canola-quality summer rape seed, oil and meal.

The development of summer rape cultivars has historically focused on the development of canola-quality open pollinated populations, but more recently the development of hybrid summer rape cultivars, particularly in Canada, Europe and Asia has been initiated. Canola-quality summer rape cultivars must be developed to meet the growing demand for oil and meal that have improved seed yield, quality, resistance to diseases and tolerance to environmental stress.

Enormous progress has been made in the development of high yielding canola summer rape cultivars and the search for even higher yielding cultivars is continuing. One of the avenues pursued to increase seed yields and improve other characteristics is to exploit heterosis available in summer rape hybrids.

High parent heterosis (20% to 70%) (Sernyk and Stefansson 1983, Grant and Beversdorf 1985, McVetty et al. 1990, Brandle and McVetty 1989, and Schuler et al.

1992) for seed yield occur in hybrids of summer rape and summer turnip rape. Significant high parent heterosis has also been reported for other agronomic characters e.g. plant height, leaf area, disease and lodging resistance (Sernyk and Stefansson 1983), and days to maturity, oil content and oil yield (Schuler et al. 1992). These observations have created interest in the development and production of hybrid summer rape cultivars to exploit the heterotic potential present in this species.

The production of hybrids, however, requires an efficient pollination control system to facilitate hybrid seed production. The more recent pollination control systems that have potential in hybrid seed production include cytoplasmic male sterility (CMS) (Kaul 1988), genetic male sterility (GMS)(Rao et al.1990), self-incompatibility (SI)(Nasrallah et al. 1991), chemically induced male sterility (Van der Meer and Van Dam 1179), genetically engineered male sterility now called nuclear male sterility (NMS)(Mariani 1990, 1991). Among these, CMS is the most extensively studied and most frequently used.

Several male sterilizing cytoplasms, which could potentially be developed into fully functional CMS systems include ogu, (Ogura 1968, Bannerot et al. 1977), nap, (Shiga et al. 1983, Thompson 1972), pol (Fu 1981). and mur (Hinata and Konno 1979) have been reported in the Brassica genus. These CMS systems all have limitations that include moderate and high temperature sensitivity (Fan and Stefansson 1986, Burns et al. 1991), lack of maintainers or restorers and biological costs (negative effects) associated with male sterile cytoplasm (McVetty et al. 1990, McVetty and Pinnisch 1994). A search for alternative CMS systems is therefore necessary.

A study was initiated in 1991 to develop and evaluate the Diplotaxis muralis (L.) DC (mur) CMS system in summer rape using a winter B. napus CMS A-line and B-line pair carrying (mur) male sterility inducing cytoplasm and its maintainer genes, respectively.

Maintainer genotypes for the mur cytoplasm were sought from a wide range of summer B. napus genotypes. The inheritance and number of genes controlling the maintenance and restoration of fertility in mur CMS was also investigated. Failure to locate any maintainer genotypes led to transfer of mur cytoplasm and maintainer genes from the mur CMS Mangun A-line and Mangun B-line into the summer rape genetic backgrounds leading to the development of three mur A-lines and three isogenic mur B-lines in summer rape.

The developed mur A-lines and B-lines were studied in greenhouse and in temperature controlled growth cabinets with particular emphasis on phenological and agronomic characteristics and temperature treatment effect. Hybrids produced between the developed mur A-lines and their respective B-lines and selected open pollinated cultivars were evaluated in four different environments in the field to investigate their comparative performance.

2. LITERATURE REVIEW

2.1 Rapeseed

Rapeseed, which belongs to the genus Brassica of the family Brassicaceae, is a common name for B. carinata, B. juncea, B. napus and B. rapa (syn B. campestris) (Bunting 1986). The cytological relationships between the four species was outlined by Morinaga (1934) who showed that B. nigra (n=8B), B. oleracea (n=9C) and B. rapa (n=10A) are the primary species and that B. carinata (n=17BC), B. juncea (n=18AB) and B. napus (n=19AC) are amphidiploids resulting from crosses between corresponding pairs of the primary species. These relationships were later confirmed by U (1935), who succeeded in artificially synthesizing B. napus by crossing the diploid species B.rapa and B. oleracea. The syntheses of B. juncea and B. carinata have been accomplished by interspecific hybridization of B. nigra X B. rapa and B. nigra X B. oleracea, respectively (Downey et al. 1975). These artificial hybridizations have provided proof for the cytogenetical relationships among the Brassica species. This understanding of the relationships among the Brassica species has facilitated gene transfer from species to species as a means of rapeseed improvement (Bunting 1986). Examples include the first double low (low erucic acid in the oil and low glucosinolate level in the meal) strain in B. rapa derived from interspecific crosses among B. rapa and B. napus (Downey et al. 1975); the transfer of genes for resistance to blackleg disease from B. juncea to B. napus (Roy 1984); and the transfer of cytoplasmic male sterility from B. rapa to B. napus (Pellan-Delourme and Renard 1987).

Rapeseed is widely cultivated for oil and meal, both derived from the seed. Cultivation is believed to have begun in Europe in the 13th century. Hougen and Stefansson (1982) stated that due to the stimulus of Government guaranteed prices, rapeseed production began in Canada in 1943.

Ethiopian mustard (B. carinata), is cultivated in north east Africa principally Ethiopia while Oriental mustard (B. juncea) is confined mainly to the Indian subcontinent and China (Kjellstrom 1993). Oilseed rape (B. napus) and oilseed turnip rape (B. rapa) are grown mainly in Europe, Canada, Australia, China and South America (Downey et al. 1975).

2.2 Canola

The commodity term "canola" is applied to varieties of B. napus and B.rapa with certain defined quality characteristics. These characteristics are: possession of less than 2% erucic acid (C 22:1) in the seed oil and less than 30 *u*mol of aliphatic glucosinolates per gram of oil free meal. Canola was developed in Canada as a means of ensuring a reliable domestic and international oilseed industry following the criticism that high erucic acid levels cause cardiac problems in mammals, and that high levels of glucosinolates yield toxic and goitrogenic cleavage products in monogastric animals. Following these quality guidelines, attention has been focused on the development of open pollinated and, canola-quality hybrid cultivars, particularly in Canada, Europe and Asia.

2.3 Hybrids

A hybrid is an F1 progeny from a cross fertilization between more or less distantly related parents that may belong to races, varieties, species or genera (Allard 1960) may arise spontaneously in nature or may be artificially induced. The development and utilization of hybrids is practised in many crop species (Kaul 1988) due to the inherent and exploitable high-parent heterosis. Commercial hybrids have been developed and utilized in many crops including corn, sunflower, sorghum and rapeseed (McVetty 1995).

2.4 Heterosis

Shull (1914) introduced the term heterosis to describe a series of complex phenomena related to higher yield and productivity in heterozygotes than homozygotes. He defined heterosis as the increase in size, yield, vigour, etc., resulting from hybridization. Allard (1960) and Simmonds (1979) defined heterosis as the converse of deterioration that accompanies inbreeding.

Other definitions of heterosis include (1) Mid parent heterosis (the improvement of the F1 hybrid over the mid parent) calculated as:[(value of F1-value of mid parent)/mid parent)x 100)] where mid parent =(value of parent 1 + value of parent 2)/2 (Falconer 1980), (2) High parent heterosis (the improvement of the F1 hybrid over the best parent) calculated as: [(value of F1-value of better parent)/value of better parent) x 100)] (Fonseca and Patterson 1968),(3) Commercial or standard heterosis (the improvement of the F1 hybrid over the standard commercially available, highest yielding non-hybrid cultivars of the day) calculated as: [(value of F1-value of standard cultivar)/value of

standard cultivar)x 100)] (Schuler et al. 1992, McVetty 1995). Heterosis has also been defined as a phenomenon of superior growth, development, differentiation and maturation caused by interaction of genes (nuclear, plastid, mitochondria), metabolism and environment (Srivastava 1983). Physiological stimulus have also been associated with heterosis (Rood et al. 1988). Four commercially important maize parental inbreds and their 12 F1 hybrids were studied to investigate the role of the phytohormone gibberellin (GA) in the regulation of heterosis. It was found that hybrids contained higher concentrations of endogenous GAs than their parental inbreds suggesting phytohormonal basis for heterosis (Rood et al. 1988)

Since the ultimate goal of plant breeding is the development of cultivars or germ plasm that are superior in all required characteristics, the discovery of heterosis or hybrid vigour, particularly in corn (Shull 1908) proved to be of great practical importance in addition to great scientific interest.

The presence of heterosis was recorded by many plant hybrids. Koeltreuter (1763) noted the early luxuriant growth of tobacco hybrids. Charles Darwin (1876) concluded that cross-fertilization was beneficial. The yield advantage associated with hybrids of corn were observed by Beal (1880). Following Beal's experiments, crosses frequently produced among cultivars exhibited yield advantage over their parents. Shull (1908), at Cold Spring Harbor Research Institute, began to self and cross-pollinate corn and noticed a striking reduction in the vigour of inbred lines. On crossing the inbred lines, however, vigour was regained.

2.4.1 Explanations for Heterosis

The earlier workers who observed heterosis did not have an explanation of the heterotic mechanism. Though there has been voluminous research on heterosis, the mechanism to date has not been fully explained or understood.

The current genetic theories put forward to explain heterosis include: 1. non-allelic or epistatic interactions (Sprague 1983), 2. allelic ("dominance" and "over dominance") interactions (Sprague 1983) and 3. intergenomic complementation (Srivastava 1983). With non-allelic or epistatic interactions, the superiority of the heterozygote is believed to be due to the regulation of one locus by another (Gowen 1952). Power (1944) found that heterosis for tomato yield results in intermediate reactions of the two non-allelic components of the fruit yield, namely, fruit number and fruit weight.

The most common theory that has been proposed to explain heterosis is that of allelic interactions. The two theories of allelic interactions are dominance and over dominance. The "dominance theory" or the "hypothesis of dominant favourable factors" Davenport (1908), Bruce (1910), Keeble and Pellew (1910) and Jones (1917) assumes that favourable factors (alleles) are dominant and deleterious factors (alleles) are recessive. Under the hypothesis, intercrossing of inbred lines lead to formation of hybrids (F1) in which the deleterious recessive alleles from one of the parents are hidden by dominant alleles in the F1 progeny. According to this theory, heterosis is caused by the complementary effects of dominant alleles at many loci. The theory was contested because if true, it should be possible to breed individuals homozygous for all the dominant factors such that heterosis could be fixed and the heterotic lines would breed

true. This has not been possible. Jones (1917) explained the difficulty by pointing out that a single linkage group would be expected to include some favourable dominant and unfavourable recessives and accumulation of dominant alleles would require precisely placed crossovers which is extremely rare. The other objection to "dominance theory" was directed at the symmetrical distributions that were observed for heterotic characters in the F2 generation. If heterosis was due to dominance, the distribution would be skewed. Jones (1917) also explained this on the basis of linkage.

The alternative "allelic interaction theory" proposed is called "over dominance". (Shull 1908) and East 1908). This theory assumes a physiological stimulus to development that increases with heterozygosity:i.e there is complementary stimulatory effect such that the heterozygote (Aa) is superior to homozygotes (AA or aa). East (1936) further explained this theory by using a divergent alleles model where a heterozygous (A_1A_2) combination of alleles at a single locus is superior to either of the homozygous combinations (A_1A_1 or A_2A_2) over a range of environments and circumstances.

In most situations, the "dominance" and "over dominance" theories lead to the same expectations. With the dominance hypothesis, the decline in vigour is expected to be proportional to decline in homozygosity and the same decline in vigour is expected with increasing homozygosity with over dominance.

The third theory of heterosis is "intergenomic complementation". It emphasizes that heterosis is regulated by interactions among nuclear, mitochondrial and chloroplast genomes rather than wholly being conditioned by nuclear genes (Srivastava 1983). It is known that a few key enzymes in the cellular energy system such as cytochrome oxidase

and in carboxylase/oxygenase in chloroplasts are jointly coded by cytoplasmic and nuclear genes. Srivastava (1983) proposed that some key parts of the cellular metabolism were co-ordinately regulated by multigenomes and hybrid vigour was due to elevation of a rate limiting step in many biochemical systems.

As reviewed above the genetic, metabolic or physiologic mechanisms of heterosis, remain to be elucidated but it is hoped that as the knowledge of plant organelles and their function develop, our understanding of heterosis will become clearer. This is important because it is the level of heterosis available for exploitation in plant and animal species that determines whether hybrids can be produced on a commercial basis. Heterosis has been evaluated and exploited in several crops including corn (Hallauer 1988), sorghum (Andrews 1987), wheat (Edwards 1987), rice (Virmani 1987), sunflower (Vranceanu 1987) and rapeseed (McVetty 1995).

2.4.2 Heterosis in Canola Rapeseed

The estimation and commercial exploitation of heterosis in other crops such as corn and wheat and particularly the recent discovery of cytoplasmic male sterility in Brassica has stimulated the interest in exploiting the heterotic potential and utilization in canola-rapeseed.

2.4.3 Heterosis for Seed Yield

Significant mid-parent, high-parent and standard heterosis for seed yield in canola-rapeseed has been known for some time. Olsson (1954), studying hybrids of a cross

between a Swedish and Japanese B. napus cultivars, found significant high parent heterosis in seed yield.

Takagi (1970) created male sterile oilseed rape plants by gamma-irradiation of the flowering plants. Hybrids produced by crossing the mutants and rapeseed cultivars showed mid-parent heterosis of 70% for seed yield. Schuster and Michael (1976) investigated the effect of inbreeding and heterosis after hand crossing of winter rape. They found that approximately one fifth of the hybrids exhibited on average 17% standard heterosis for seed yield.

Shiga (1976) evaluated the performance of 131 hybrids produced using cytoplasmic male sterile plants and various pollinators and obtained mid-parent heterosis of 40% for seed yield. He also found that general combining ability was more important than specific combining ability in controlling heterosis in the inbred lines.

Buson (1980) evaluated the performance of inbred lines and their F1 progenies in an incomplete diallel experiment involving 25 winter oilseed rape (B. napus L.) lines. He also assessed the relative importance of general and specific combining ability. A total of 130 hybrids were obtained. The combinations were made on the consanguinity of the inbred lines. The hybrids showed 23% high-parent heterosis for seed yield.

Guan (1980) studied the potential for exploitation of heterosis using 8 intercultivar hybrids and 11 hybrids of male sterile lines crossed with fertility restoring cultivars of oilseed rape (B. napus) in China and observed high-parent heterosis of up to 60% for seed yield. He also determined stomatal number per unit area of siliqua epidermis, flowering time, leaf area index, chlorophyll content, photosynthetic rate, and exuding water rate for

the hybrids and parents. He found that the hybrids were superior to the parents for all these characteristics suggesting that the hybrids have a greater photosynthetic capacity as the basis for their heterosis which is expressed in vigorous growth and high seed yields. This indicated that development of higher photosynthetic capacity would lead to higher exploitable heterosis in F1 hybrids.

To discover whether a comparable level of high-parent heterosis for seed yield could be obtained from hybrids of summer rape (B. napus) in western Canada, Sernyk and Stefansson (1983) produced hybrids between Asian or European varieties top crossed to the Canadian cultivar Regent. The seed yields from hybrids between Marnoo and Regent and Karat and Regent showed high-parent heterosis of 38% and 43% respectively, for seed yield.

Olivieri and Parrini (1983) in France, evaluated 420 single cross hybrids from a diallel cross of 20 winter and spring rape cultivars. The combinations among winter and spring cultivars showed the highest heterosis for seed yield. General combining ability effects were more important than specific combining ability indicating that additive genetic effects controlled the expression of seed yield.

Grant and Beversdorf (1985) investigated high-parent heterosis and combining ability estimates for hybrids produced by intercrossing high yielding Canadian and European spring planted cultivars of oilseed rape (B. napus L.). Hybrids exhibited positive high-parent heterosis of up to 72% for seed yield in the crosses. Specific combining ability was more important than general combining ability for seed yield, indicating that heterosis in seed yield is controlled by non-additive genetic effects. The cultivars Topas

and Regent were the best general combiners for seed yield. The best specific combinations for seed yield heterosis, Westar x Hanna, Regent x Line and Regent x D-1, exhibited average high-parent heterosis of 50, 38 and 30% for seed yield respectively.

To investigate the performance of hybrids produced using parental sources from the same and different geographical regions, Buson (1987) produced hybrids among and between European and Asiatic inbred lines of oilseed rape. The results of the experiments showed the superiority of European x Asiatic hybrids for seed yield in relation to all other cultivars, selfed lines and European or Asiatic hybrids. The yielding ability was, however, shown to depend on the year, environment and lines used in crossing. The experiments indicated that there is higher heterotic potential in hybrids derived from parents of diverse genetic background as estimated by geographic origin of the parents. Brandle and McVetty (1989) conducted a trial consisting of 32 B. napus entries; 18 inbred line derived hybrids and their 9 inbred line parents and 2 cultivar derived hybrids and their parents. The results showed that some inbred-line derived hybrids had significant high parent heterosis of up to 120% for seed yield. There were also differences in general and specific combining ability among inbred lines indicating that the presence of variability in breeding values among cultivar derived inbred lines and hybrid oilseed rape breeding programs should be based on inbred line crosses rather than cultivar crosses. General combining ability was found to be significant and accounted for 88% of the cross sum of squares while specific combining ability was non-significant indicating that additive genetic effects predominantly influenced the expression of yield in this experiment.

Schuler et al. (1992) evaluated the potential for heterosis utilization in summer turnip rape in western Canada using hand crossed hybrids between B. rapa L. var. oleifera cv Tobin and 19 European and Canadian strains and observed average mid-parent heterosis of 18%, high-parent heterosis of 12% and commercial heterosis of 24% for seed yield. Falk et al.(1994) evaluated the performance of intercultivar summer turnip rape hybrids produced in the greenhouse from reciprocal crosses between three genetically different Canadian cultivars Echo, Torch and Tobin and the Swedish strain Sv8236580. They observed an average of 13% mid-parent heterosis and high parent heterosis of up to 27% for seed yield. Falk et al.(1994) also evaluated the performance of single cross hybrids of summer turnip rape in Saskatchewan and observed mid-parent heterosis of 35% for seed yield. From the studies, they found that heterosis for seed yield was greatest in crosses between genetically diverse cultivars which agrees with classical theories of heterosis.

2.4.4 Heterosis for Growth Characters

Significant heterosis in rapeseed has been demonstrated for a number of characters other than seed yield. Olsson (1954), studied hybrids of a cross between Swedish and Japanese B. napus cultivars and found significant heterosis over the parents in plant height. Schuster and Michael (1976) investigated the effect of permanent inbreeding and heterosis after hand crossing of winter rape and found mid-parent heterosis of 14% for plant height. Shiga (1976) also evaluated heterosis levels in hybrids made between Japanese and European winter rape (B. napus L.) cultivars. Mid-parent heterosis was

observed for length of inflorescence and number of primary branches. The evaluation of performance by Shiga (1976), of 131 hybrids produced using cytoplasmic male sterile plants showed mid-parent heterosis for leaf area, plant height, number of pods per plant and number of seeds per pod. General combining ability was more important than specific combining ability in controlling the expression of heterosis in the inbred lines.

Buson (1980) evaluated the performance of hybrids in an incomplete diallel experiment involving 25 winter rape (Brassica napus L.) lines. She also assessed the relative importance of general and specific combining ability. A total of 130 hybrids was obtained. Mid-parent heterosis was expressed on characters connected with vegetative development (leaf area, plant height) and yield components (number of pods and seed per pod).

Guan (1980) studied heterosis levels using 8 intercultivar hybrids and 11 hybrids of male sterile lines crossed with fertility restoring cultivars of rape (B. napus) in China. He found significant mid-parent heterosis in the number of primary branches and number of siliqua per plant.

Sernyk and Stefansson (1983) produced hybrids between Asian or European varieties top crossed to the Canadian cultivar Regent, and observed high-parent heterosis for both total dry matter and harvest index.

Grant and Beversdorf (1985) investigated heterosis and determined the combining ability estimates for hybrids produced by intercrossing high yielding Canadian and European spring planted cultivars of oilseed rape and found that heterosis for plant height and lodging resistance was nonsignificant. Specific combining ability was more important

than general combining ability for plant height and lodging resistance, indicating that heterosis in these characteristics is controlled by nonadditive genetic effects.

2.4.5 Heterosis for Quality Traits

Heterosis for quality traits has also been investigated in canola rapeseed. In summer turnip rape, about 52% mid-parent and 34% high-parent heterosis has been reported for oil content (SwamyRao 1970). Schuler et al.(1992) found that hybrids made between B.rapa cv Tobin and 19 European and Canadian strains exhibited mid-parent heterosis of -1.0% for oil content and 17% for oil yield. Falk et al.(1994) evaluated the performance of inter-cultivar summer turnip rape hybrids made between Canadian cultivars Echo, Tobin and Swedish strain Sv823680 and found high-parent heterosis of as low as -4.7% for oil content. In canola rapeseed, fluctuations between protein and oil content have been reported (Brandle and McVetty 1989). An increase of 0.8% in oil and a reduction of 0.5% in protein were reported in hybrids developed from inbred B. napus lines in the nap cytoplasm. Grant and Beversdorf (1985) reported little or no high parent heterosis for oil content, and negative high-parent heterosis for protein content in oilseed rape hybrids.

2.4.6 Heterosis in Canola Rapeseed - a Summary

In summary, significant mid-parent, high-parent and standard heterosis for seed yield has been frequently observed in canola rapeseed. In contrast, evidence for mid parent, high-parent or standard heterosis for seed quality traits in canola rapeseed is

limited. Most of the investigations have been on intercultivar-cross-derived hybrids. However, it has also been shown that inbred-line-cross-derived hybrids exhibit higher high parent heterosis (up to 120%) for seed yield. General combining ability and hence additive genetic effects account for the expression of heterosis. Hybrids from parents from different geographical regions generally have a higher heterotic expression than from parents of the same geographic origin. However, the development of hybrids and utilization of the inherent heterosis requires an efficient pollination control mechanism.

2.5 Pollination Control Systems

pollination control systems that have potential applicability in hybrid seed production include, hand emasculation and pollination, gametocides (chemically hybridizing agents (CHA)(Van der Meer and Van Dam (1979), genetic male sterility (GMS)(Rao et al. 1990), self-incompatibility (SI)(Nasrallah et al.1991), and genetically engineered male sterility (nuclear male sterility- NMS) (Mariani et al. 1990, 1991, Williams, 1995) and cytoplasmic male sterility (CMS) (Kaul 1988).

2.5.1 Hand emasculation and pollination

The earliest method used to produce hybrids, particularly in corn, after the discovery of exploitable heterosis was by hand. This method involves the manual control of pollination by emasculation of flowers prior to anthesis followed by transfer of pollen from anthers to stigma (Poehlman 1979). Manual emasculation in corn is practical since the monoecious morphology of the plants permits easy emasculation prior to anthesis by

removal of tassels (Welsh 1982). In contrast, in perfect flowered plants such as canola rapeseed, manual emasculation is not practical for commercial scale hybrid seed production. Hybrid seed production by manual emasculation is time consuming and necessitates use of considerable labour. Human error may also make the method inefficient in terms of hybridity (proportion of hybrid seed in a hybrid seed lot). The hybrids produced by hand pollination are not economically practical and this prompted the search for less expensive and more reliable pollination control methods to permit economic production of hybrid seed (Fang and McVetty 1989).

2.5.2. Gametocides

The use of gametocides (chemical hybridizing agents) could be an alternative to the use of genic or cytoplasmic male sterility as a pollen control method (Williams, 1995). The chemical sterilization of the pollen producing organs would eliminate emasculation. The method involves the use of foliar spray before flowering that inhibits production of viable pollen, but does not injure the pistillate production organs (Poehlman 1979, Welsh 1982). The past work has revealed some problems. The major problem has been the failure to obtain complete pollen sterility due to variability in response under different environmental conditions. Determination of the critical stage of plant development and correct application rate has also been a problem. The indeterminate flowering nature of the Brassica suggests that gametocides will be of questionable value for the commercial production of hybrid seed (Downey and Rimmer 1993). In Brassica limited work has been reported on the use of gametocides. Van der Meer and Van Dam

(1979) were able to maintain some plants of B. oleracea cultivars in a completely male sterile condition for up to 24 hours by spraying repeatedly with varying concentrations of GA 4/7 in isopropyl alcohol. However reversion to complete male fertility occurred a few days after treatment. Therefore, though the chemical gametocides have potential, its applicability has yet to be perfected.

2.5.3. Genic male sterility

Genic male sterility system though not as widely used as the CMS can also be utilized in production of hybrids. It is manifested through nuclear genes inhibiting the normal development of anthers and pollen. It is predominantly conditioned by a pair of recessive alleles (Rao et al. 1990. The recessive alleles can be introduced into a line through crossing, selfing and selection. A pure population of genetic male sterile plants can not be produced but male steriles may be carried along at high frequency in a self pollinating crop if seeds from the male sterile plants are harvested and used to plant the next generation. To maintain the sterile plants, the sterile plants are crossed with heterozygous male sterile plants. The progeny of the cross segregates into 50% male sterile and 50% male fertile (Poehlman 1979). The fertile plants must be removed before flowering. The sterile plants can then be crossed with selected male inbred lines to make the hybrid. The system is thus more complicated and is used where CMS is not available or where there is a problem with the CMS system.

In Brassica napus, Takagi (1970) obtained a monogenic recessive male sterile mutant through the gamma-ray irradiation of oilseed rape plants. Male sterile plants were

easily distinguishable from normal plants in the flower stage or during early flowering. The plants were used to produce F1 hybrids. A diallelic epistatic system was developed and is being commercially utilized in China (Lee and Yan, 1983, Li et al. 1988). Pollen production in this system is controlled by two loci with epistatic effects. In B. rapa and and yellow sarson a number of monogenic recessive male sterile mutants have also been reported (Chowdhury and Das 1967, Zuberi and Zuberi, 1983).

2.5.4. Self-incompatibility (SI)

Self-incompatibility (SI) can be used to produce hybrid progeny (Fu et al. 1995). The incompatibility is a form of infertility caused by the failure of plants with normal pollen and ovules to set seed. The incompatibility prevents self-fertilization and fosters cross fertilization (Poehlman 1979). The self-incompatibility occurs widely in flowering plants including the Brassicaceae. It is classified into two groups, gametophytic and sporophytic self-incompatibility. In Brassicaceae the SI system is the sporophytic type (Hinata and Nisho 1980)

In plants that exhibit self-incompatibility the interaction between the pollen and the pistil lead to the inhibition of self-pollen and hence the failure to set seed (Nasrallah and Nasrallah 1989, Nasrallah et al. 1991). In many species the genetic control of self-incompatibility is not only mediated by a single locus called the "S" locus with multiple alleles but also by alleles at a complementary locus (Hinata and Okazaki, 1985). It has been proposed that identical S alleles products, the glycoproteins in the pollen and the stigma or the style, associate and form dimer or tetramer complexes which trigger the

rejection of pollen by the female tissue (Clarke et al. 1990). So, for compatibility to occur, there has to be different alleles in the pollen and stigma.

The self-incompatibility can be used to produce hybrids but the incompatibility has to be overcome in order to produce the SI inbreds. This is accomplished by bud pollination, rupturing the stigmatic surface, increasing CO_2 concentration (poehlman 1979, Ito 1981,) In B. napus SI hybrids of spring canola have been registered in Canada. The first SI hybrids were produced using a system patented in Canada by Kingroup Inc. (Scott-Pearse, 1991) that involves using microspore culture to produce doubled haploid plants that are homozygous for either SC (self-compatibility) or SI alleles. When SI plants are pollinated with pollen from SC plants a heterozygous self-incompatible parent is produced. This SI parent is used as the female in hybrid seed production with an SC line serving as the pollen parent.

2.5.5. Nuclear male sterility

Genetically engineered male sterility production methods have been proposed or are being used to produce hybrid varieties. (Williams, 1995). Male sterility has been constructed through chimeric ribonuclease genes. The expression of these genes lead to destruction of the tapetum resulting in malfunctioning of anthers and the consequential production of male sterile plants. Normal pollen is inhibited by linking pollen specific promoters to genes expressing the chimeric ribonuclease genes or the antisense genes. Mariani (1990,1991) genetically engineered male sterility in oilseed rape (B. napus). Two dominant nuclear genes that interfere with the functioning of CMS, RNase T1 from

Aspergillus oryzae and Barnase from Bacillus amyloliquefaciens were combined with a promoter from tobacco that expresses only in the tapetum cells of immature anthers. The transfer of these genes resulted in male sterile plants with normal flowers and growth habit. A fertility restorer gene, Barstar was also constructed from Bacillus amyloliquefaciens. The barstar gene encodes a protein that inhibits the activity of Barnase and RNase T1. When the B. napus plants transformed with Barstar were crossed with the male sterile Barnase plants the progeny was male fertile showing that the genetically engineered male sterility can also be restored with genetically engineered fertility restorer. The maintenance of the female parent requires backcrossing to a non-transformed maintainer resulting in a 1:1 ratio of male sterile to male fertile plants. To overcome this problem, a construct was built into a vector containing the marker gene neo (coding for neomycin phosphotransferase II) conferring resistance to the antibiotic (kanamycin) and Bar (coding for phosphinotricin acetyl transferase) conferring resistance to the herbicide phosphinotricin. The marker genes were used for selection. The female parent is maintained by backcrosing to the non transformed maintainer parent and the male fertile herbicide-susceptible segregates are removed in the seedling stage through the application of the herbicide. Commercial F1 seed is produced from fields containing the male fertile parent homozygous for the Barstar and herbicide resistant genes and the male sterile female heterozygous for Barnase and herbicide resistance. The F1 progeny are male fertile and herbicide resistant. This system is under extensive evaluation in Canada (Downey and Rimmer 1993).

2.5.6 Cytoplasmic Male Sterility Systems

Sterility is a condition that is characterized by non-functional gametes caused by chromosomal aberrations, gene action or cytoplasmic influence that cause abortion or modification of the entire flower, stamen or pistil and upsets the development of the pollen embryo sac or embryo, (Poehlman 1979).

Cytoplasmic male sterility (CMS), controlled by genes in the cytoplasm and influenced by nuclear genes, is the inability of higher plants to produce functional pollen (Poehlman 1979, Gregory et al. 1985). CMS is used as genetic form of emasculation to facilitate the making of hybrids and hence reduce the high costs. It is the most widely used pollination control system for large-scale production of hybrids in many crops, including corn (Pearson 1981), sunflower (Leclercq 1969) and canola rapeseed (McVetty 1995). Cytoplasmic male sterility systems utilize two alternative cytoplasms. These are, firstly, the sterility-inducing cytoplasm called "S" (sterile) cytoplasm and secondly, the non-sterility inducing cytoplasm called normal or "F" (fertile) cytoplasm. Male-sterility is conditioned by nuclear genes with at least two allelic forms: *rf* (recessive) and *Rf* (dominant). Male sterility is produced by the homozygous recessive genotype (*rf rf*) in the sterile cytoplasm, while male fertility is produced when the cytoplasm is the normal type or when the nuclear genotype is *RfRf* or *Rfrf* in either the normal or sterile cytoplasm. Cytoplasmic male sterility occurs widely in the plant kingdom (Edwardson 1956, 1970, Kaul 1988) and is found in many crops, including canola-rapeseed (McVetty 1995).

2.5.6.1 Cytoplasmic Male Sterility in Brassica Species

Cytoplasmic male-sterility has been discovered in several Brassica species in the last three decades and there are several male-sterility inducing cytoplasms currently under development for use in canola-rapeseed.

2.5.6.1.2 nig CMS. Pearson (1972) treated F1 plants of Brassica nigra (L. Koch) (black mustard) x B. oleracea L.(broccoli) with colchicine to produce the amphidiploid. Using a repeated backcross procedure, the genome of B. oleracea was transferred into the cytoplasm of B. nigra. From the outcrosses with cabbage, cytoplasmically-inherited male sterile plants were isolated. The sterility was expressed only in the B. nigra (nig) cytoplasm. In the sterile plants, the stamens were reduced to petal or petal-like structures and the nectaries were lacking. This CMS system was designated nig by Shiga (1980).

2.5.6.1.2 oxy CMS. Prakash and Chopra (1988) obtained oxy CMS plants by placing the genome of B. rapa spp oleifera into the cytoplasm of B. oxyrrhina through a repeated backcross technique. Male sterility in the system was very stable and the flowers showed no abnormalities except that the anthers were small, slender, non-dehiscent and contained non-functional pollen. Some chlorosis of the first leaves of male sterile plants was noted but this has been overcome through protoplast fusion. Gene(s) for fertility restoration have been transferred from oxyrrhina and if the restorer proves to be effective, the system will have a high potential for development.

2.5.6.1.3 ogu CMS. The ogu male sterility inducing cytoplasm was initially discovered in radish (Raphanus sativus L.) by Ogura in 1968. Bannerot et al.(1977) transferred the nucleus of B. napus into the male sterile radish cytoplasm through intergeneric crossing followed by back crossing to B. napus. Shiga (1980) classified the male-sterility inducing cytoplasm in B. napus as the ogu cytoplasm. The utilization of ogu system was initially limited by chlorosis at low temperatures (below 12^0C), flowers unattractive to pollinators due to the absence of nectaries and the lack of restorers for this cytoplasm (Rouselle et al. 1984).

The above deficiencies were overcome by protoplast fusion resulting in production of male sterile non-chlorotic plants with well developed nectaries (Pelletier et al. 1983, Pelletier 1990). The problem of restoration has been solved by backcross breeding (Delourme et al. 1991) resulting in ogu CMS system with monogenic restorers without impaired female fertility, making the system potentially functional in B.napus (Renard et al. 1992).

2.5.6.1.4 nap CMS. The nap cytoplasmic male sterility system was first reported in rapeseed by Shiga and Baba (1971,1973) and Thompson (1972). Shiga and Baba (1971, 1973) reported that male-sterile plants were observed in F2 generation of a cross between Chisaya-natane used as female and Hokuriku 23. The reciprocal cross showed that the male sterility was based on a sterility inducing cytoplasm. Thompson (1972) crossed a number of winter and spring cultivars with the Polish cultivar Bronowski and male sterile plants were found in F2 generation. Shiga (1976) and Shiga et al. (1983) compared the

fertility restoration pattern of the two systems discovered by Shiga and Baba (1971) and Thompson (1972) and found that the fertility restorers and maintainers were basically the same indicating that they possessed the same type of male sterile cytoplasm of B. napus. Shiga (1980) designated the cytoplasm as nap CMS. Studies of the nap system involving Bronowski revealed that it carried the recessive *rf* allele for male sterility but a male fertility conditioning (F) cytoplasm. All the female genotypes carried the dominant restorer allele (*Rf*) and a sterility inducing (S) cytoplasm.

2.5.6.1.5 pol CMS. Another cytoplasmic male-sterility inducing cytoplasm is the pol cytoplasm reported by Fu (1981). He found that male sterile plants occurred spontaneously in the B. napus cultivar "Polima", that originated from Poland. The male sterility was thus designated as pol CMS. Most summer rape strains tested in Canada at least partially maintain the sterility and three dominant genes for fertility restoration are available in B. napus. Fan et al. (1986) developed a B. napus restorer line for the pol cytoplasm using interspecific crosses to a zero erucic acid mustard (ZEM) B. juncea line. Fang and McVetty (1989) found two genetically different pol CMS restorer sources, the cultivar "Italy", and the University of Manitoba accession "UM2353". The two restorer sources provide adequate male fertility restoration of the pol CMS system for commercial use in hybrid canola production.

2.5.6.1.6 ctr CMS. The ctr system was initially observed in crosses between triazine-resistant lines of B. napus cv Tower and the low glucosinolate cultivar "Bronowski"

(Grant 1984). Male sterility in the cytoplasm is both "deep" and "stable". Two to five restorer genes have been identified in the canola-rapeseed cultivars "Westar" and "Tower" but maintainer lines have not been developed to date.

2.5.6.1.7 mur CMS. The Diplotaxis muralis male sterile cytoplasm and derived CMS system termed mur in B. rapa was developed from Diplotaxis muralis.(L.) DC. D. muralis (sand rocket) is a short lived weed that occurs in annual, biennial or perennial forms and is mainly distributed in southern and central Europe (Tutin et al. 1964) and in areas surrounding the Mediterranean. It is an allotetraploid species (n=21) derived from D. tenuifolia (n=11) and D. vinimea (n=10) (Harberd and Mcarther 1972). The sterility inducing cytoplasm has been used to develop male sterile lines in B. rapa (Hinata and Konno 1979) and B. napus (Pellan-Delourme and Renard 1987).

Hinata and Konno (1979) treated the F1 plants of D. muralis x B. rapa variety Yukina with colchicine to produce the amphidiploid (4n) and reduced the number of chromosomes to the diploid level (2n) by repeated backcrosses with Yukina pollen, thus placing the B. rapa genome in the D. muralis cytoplasm. From the progenies of these backcross populations, a male sterile line inherited cytoplasmically was isolated which expressed sterility only in the mur cytoplasm. The nuclear genotype of this male sterile line was *rfm rfm*. The mur cytoplasm induced male sterility was characterized by two nectaries, narrow petals, and short filaments with non-dehiscent anthers that contained a small amount of pollen. The sterility was considered to be controlled by both nuclear genes and the cytoplasm originally from D. muralis. Shiga (1980) observed that F_1

hybrids between D. muralis and B. napus (Norin 16) were male sterile and designated the male sterility as the mur CMS. He also indicated that no restorer gene for the mur CMS was available in B. napus strains used in the experiment. However, further experiment by Pellan-Delourme and Renard (1987) found that the crosses between cytoplasmic male sterile B. rapa with D. muralis cytoplasm and Norin 16 produced male-fertile plants indicating that Norin 16 carried restorer genes for D. muralis cytoplasm.

The mur cytoplasm was also used to produce sterile plants in B. napus Fan et al. (1985) used the Canadian cultivar Regent in a backcross series into mur cytoplasm. The back crossed progenies showed less than 20% of male-sterile plants. Sterile plants from the sixth generation of back crossing were crossed with twelve cultivars of oilseed rape. The resulting progenies showed 0 to 36% sterile plants but a segregation ratio could not be defined.

A cytological examination of the progenies revealed that all the sterile plants were carrying a single supernumerary chromosome while the chromosome number of the fertile plants was normal. On the basis of these results it was concluded that the sterility was associated with the extra chromosome, probably from D. muralis (Fan et al. 1985).

Fertility restoring genes for mur cytoplasm were found in B. rapa (Hinata and Konno 1979). All Canadian and European B. rapa cultivars tested restored the male fertility induced by D. muralis cytoplasm (Fan et al. 1986). Experiments by Pellan-Delourme and Renard (1987) in search for maintainer genotypes revealed that most of the B. napus genotypes carry restorer genes for mur cytoplasm. Though restorer genes for mur cytoplasm have been identified, in B. napus, maintainer genes have not been found

in summer cultivars of this species and the identification of maintainer genes in summer B. napus, or the transfer of maintainer characteristics from B. rapa cultivar Yukina or some other source into summer B. napus is essential for the development of a D. muralis based CMS system in summer B. napus (Downey and Robbelen 1989).

2.5.6.2 CMS Origin and Mechanism. Cytoplasmic male sterility, due to incompatible nuclear-cytoplasmic interactions, (Hanson and Conde 1985) is a maternally inherited trait that arises from intergeneric crosses, interspecific crosses, intraspecific crosses and from the action of mutagens or antibiotics on cytoplasmic genes (Edwardson 1956, 1970). Extensive studies have established that CMS results from the altered properties of mitochondria (Mahipal and Gregory 1991), but the mechanisms are not fully understood (McVetty 1995).

Pollen development takes place in the anther. The anther has four wall layers, the epidermis, the endothecium, the middle layer and the tapetum enclosing the fluid filled locule that contains sporogenous cells which develop into pollen grains. The layer of anther cells adjacent to the locule is known as the tapetum and it is the tissue that is intimately involved in microsporogenesis (Bedinger 1992). It has been proposed that the tapetum provides enzymes and nutrients for microspores production (Pacini et al. 1985, Scott et al. 1991 Bedinger 1992,). One important function of the tapetal cells is to release the haploid microspores through the production of an enzyme *B*eta 1-3 glucanase (callase), that hydrolyses the callose wall (Scott et al. 1991). The timeliness in the secretion of the enzyme is critical for pollen development (Izhar and Frankel 1971).

Another role of the tapetum is in the production of structural compounds (sporopollenin) that make the outer layer of the pollen grain i.e the exine (Scott et al. 1991). Therefore, the involvement of tapetum in nutrition, energy supply, structural functions and microspore release are among the important roles in pollen development and thus premature destruction of the tapetum leads to male sterility. The failure of the tapetum to perform normal and necessary functions is a commonly presumed cause of CMS in many plant species (Edwardson 1956, 1970, Kaul 1988).

In oilseed rape (B. napus), Fan (1985) reported that failure of microsporogenesis for both nap and pol CMS was associated with lack of differentiation of the first sporogenous cells before the archesporial stage of pollen development. Fan (1985) and Polowick and Sawhney (1990) reported that the abortion of microspore in ogu CMS may be the consequence of abnormal vacuolation of the tapetum.

Grant et al. (1986) reported that the development of male sterility in the ctr cytoplasm of summer oilseed rape (B. napus) was similar to that of male fertile anther until the end of prophase I. Following this stage in the CMS, the microspores degenerated within the callose walls and the tetrad stage was not reached. The degeneration was found to occur simultaneously with the proliferation of the tapetum which eventually fills the anther locule.

CMS has been associated with the mitochondrial genome (Kaul, 1988, Andre et al. 1995). It has been assumed that the abnormal behaviour of the tapetum is due to defective mitochondria since it has been well established that the genetic determinants of CMS reside in the mitochondria and the nuclear genes control its expression (Newton

1988). Singh and Brown (1991), Handa and Nakujima (1992) and Handa (1993) reported that pol CMS mitochondria could be distinguished from normal mitochondria using restriction endonuclease analysis. They reported that the difference between the normal and the pol CMS mitochondrial genome is that the pol CMS mitochondrial genome contains an unidentified reading frame (urf) upstream atp6 gene that is co-transcribed with atp6 gene. A 1100 nucleotides long mitochondrial (mt) RNA transcript was detected in both pol CMS and normal mitochondria but a 2100 nucleotides long transcript was only present in pol CMS RNA. Witt et al. (1991) postulated that the transcript is altered by the restorer genes. These observations indicated that pol urf is directly involved in the defects that lead to male sterility in pol CMS lines.

Handa and Nakujima (1992) postulated two functional models for the action of pol urf. In the first model, the pol urf protein (ORF B) and unknown peptide might act as antagonist to functional ORF B in the mitochondria. In the second model the presence of the 2100 nucleotides long transcript may interfere with normal atp6 mRNA functions either by limiting availability of the 1100 nucleotides long transcript at atp6 by necessitating a processing step or interfering with normal transcription. Transcription of mitochondrial gene (orf138) has been associated correlated with CMS in rapeseed cybrids (Renard 1995). Mitochondrial chimeric genes have also been observed and associated with cytoplasmic male sterility in corn (Dewey et al. 1987, Forde, Leaver 1980, Levings 1993), petunia (Hanson 1991), Sorghum (Bailey-Serres et al. 1986) and sunflower (Kohler et al. 1991, Christina et al. 1994)

2.5.6.3. Explanation of CMS Mechanism. Mitochondria found in the tapetal layer are the centres for energy metabolism. The mitochondrial genes encode polypeptides that are components of the electron transport system (complexes I, II, III, and IV), and the mitochondrial protein synthesis system (Eckenrode and Levings 1986, Newton 1988). They also code for structural RNA's such as 26S 45S rRNA's and for tRNA's. Mitochondrial gene mutations are predicted to directly affect the electron transport system and ATP formation or RNA translation and thus possibly affecting or blocking pollen formation resulting in CMS (McVetty 1995). Singh and Sawhney (1995) from their experiments with ogu CMS suggested that male sterility may be associated with altered levels of plant hormones, viz. cytokinins (CK) and abscisic acid (ABA). is

2.5.6.4.Maintenance of CMS Systems. The maintenance of male sterility for ogu, pol and nap CMS systems was evaluated by Fan et al. (1986). Thirty two B. napus strains were crossed to male sterile plants carrying one of the three cytoplasms. All strains were found to be maintainers for the ogu CMS system. Male fertility for the nap CMS system was fully restored by 30 strains. A few of the strains were maintainers for the pol CMS system, while most were partial maintainers for the pol CMS system.

Pellan-Delourme and Renard (1987), investigating the maintenance of mur CMS, found that F1 plants obtained from crosses with B. napus cultivars Mangun and Hinchu were male sterile, indicating that the two cultivars carried maintainer genes for the mur CMS system. The two cultivars are, however, of Asiatic origin. Since many Asiatic B. napus cultivars are derived from crosses between B. napus and B. rapa (Shiga 1980), it

is possible that the presence of maintainers in Mangun and Hinchu result from this interspecific hybridization and not directly from B. napus. Attempts by Fan et al. (1986) to obtain a mur CMS maintainer in the summer canola rapeseed B. napus cultivar Regent were not successful; indicating that Regent does not carry maintainer genes for mur CMS.

2.5.6.5 CMS System Limitations. Nearly all CMS systems in the Brassicas have limitations that make them difficult or impossible to use as pollination control systems in the production of commercial quantities of hybrid seed. These limitations range from pleiotropic negative effects of the CMS on the agronomic performance or quality, to complex and environmentally unstable maintenance of male sterility and fertility restoration, to inability to produce commercial quantities of hybrid seed because of poor floral characteristics for cross pollination (McVetty 1995).

2.5.6.5.1 nap CMS Limitations. The effect of temperature treatment on the expression of male sterility in nap CMS was studied by Fan and Stenfansson (1986). They grew plants in the greenhouse and transferred them to temperature regimes of 22/16, 26/20 and 30/24^{0}C for seven days followed by transfer back to growth rooms at 22/16^{0}C and then examined anther type and stamen length every two days. Flowers were classified as either male-sterile, partially male-sterile or male-fertile based on the degree of anther development. They found that male-sterile and partially male-sterile plants remained stable at 22/16^{0}C, but at 26/20^{0}C the male-sterile plants showed some pollen production while the partially male-sterile plants were fully male-fertile. At 30/24°C, there was a reversion

to partial and full male-fertility by the male-sterile and partially male-sterile plants respectively showing that nap system in oilseed rape is unstable at moderate to high temperature and therefore, not commercially viable (Fan and Stefansson 1986).

The high temperature reversion/sensitivity results in incomplete male sterility resulting in selfing and sibling that reduces the hybridity of field-produced hybrid seed lots (Pinnisch and McVetty 1990). The usefulness of the nap system is also limited since 3 to 5 genes may be involved in fertility restoration (Sernyk and Stefansson 1983).

2.5.6.5.2 pol CMS Limitations. Fan et al. (1986) reported that pol CMS is also temperature sensitive. They found that pol CMS-A lines were completely male sterile at 22/16^{0}C and 26/20^{0}C but reverted to partial male sterility at temperatures over 30^{0}C. The temperature sensitivity and male sterility reversion results in selfing and sibing that reduces the hybridity (proportion of hybrid seed) of the field produced hybrid seed lots (Pinnisch and McVetty 1990)

Burns et al. (1991) also studied the effect of high temperature treatment on the expression of pol cytoplasmic male sterility in summer rape. They evaluated the nuclear genotypes from each of the cultivars Karat, Marnoo and Regent for the ability to maintain pollen sterility in the pol cytoplasm under controlled environment and in the field. Seven days of exposure to day/night temperatures of 30/24°C in controlled environment led to increased pollen production in all the F1 populations in the study. Maximum reversion to male fertility occurred 6 to 13 days after removal from the high temperature treatment. The study showed that there was linear relationship between daily

mean temperatures in the field and mean male sterility index (MSI). The observed variation in the stability of male sterility in the F1 indicated the presence of different maintainer genes among the inbreds of each cultivar. The study concluded that until the temperature stability of pol CMS is improved, close observation of long term climatic conditions will be required to select the growing areas best suited for pure hybrid seed production and consequently the utilization of heterotic potential of the germ plasm with the pol cytoplasm. Studies of fertility restoration by high temperature in the ogu CMS showed that the ogu male sterility is stable and is not influenced by high temperature (Fan, 1985, Polowick and Sawhney 1988). The phenomenon of temperature sensitivity has also been observed in other crops for example, cotton (Marshall et al. 1974), corn (Tracy et al. 1991) and Petunia (Marrewijk 1969).

2.5.6.6 Biological Cost of CMS. Cytoplasmic male sterility arises from imbalanced or negative nuclear cytoplasmic interactions (Edwardson 1956, 1970). The nuclear interactions not only produce the male sterility but are also responsible for other associated negative effects on the agronomic performance of genotypes present in the sterilizing cytoplasm (Hanson and Code 1985, McVetty 1995).

In a comparative study of 3 male fertility restored hybrids made in both nap and pol male-sterility-inducing cytoplasms conducted in 5 environments over 2 years the hybrids in pol cytoplasm were found to yield on average 23% less than the same hybrids in the nap cytoplasm (McVetty et al. 1990). The same workers also found that the hybrids in pol cytoplasm were on average 3% lower in relative oil content than nap

hybrids. There was no evidence of high-parent heterosis for oil content or protein content for either nap or pol hybrids. The nap hybrids displayed on average, 70% high parent heterosis for yield while the pol hybrids displayed on average 31% high-parent heterosis for yield. From the studies, McVetty et al. (1990) concluded that there is a biological cost associated with the use of the pol cytoplasm. but the cost was not, however, so high as to eliminate the heterotic advantage of hybrids made in the pol cytoplasm as compared to the better parent used in the cross indicating that the pol cytoplasm, although not entirely desirable, can be used to make canola rapeseed hybrids until such a time as a better alternative is found.

McVetty and Pinnisch (1994) evaluated the effects of nap and pol cytoplasms on the performance of three oilseed derived isoline pairs. They reported that one nap line yielded significantly more seed (17%) than the corresponding pol line, three nap lines had significantly higher protein content (2 to 3% more relatively) than their corresponding pol lines and two nap lines produced significantly more seed energy (4 to 18% more relatively) than their corresponding pol lines. It was concluded that there are pleiotropic negative effects (biological costs) associated with the pol cytoplasm and the negative effects are affected by nuclear genotypes and appear to be related to the "depth" of male sterility.

2.6 Correlations Among Seed Yield, Biological Yield, Harvest Index and Seed Quality

Increasing seed yield is one of the important objectives in rapeseed improvement. Seed yield is often related to a combination of classical yield components. The number of plants per unit area, number of pods per plant, number of seeds per pod and seed weight (Thurling 1974 a). Other plant characteristics often associated with increased seed yield include vigorous growth, rapid and strong rosette formation, deep root penetration, resistance to lodging and shattering, and resistance to diseases (Downey and Rimmer 1993)

Seed yield and seed quality characteristics have been investigated for high-parent heterosis. Since seed yield is probably the most difficult trait to measure accurately, numerous attempts have been made to identify the most important components required to produce high seed yields. Seed yield itself is the product of biological yield and harvest index (Donald 1962). Biological yield is the total dry matter (TDM) above the ground. Harvest index is the seed yield expressed as percent of biological yield or TDM. Ideally the biological yield used to compute harvest index should include all above and below ground structures, but for practical reasons, only the above ground structures remaining at maturity (i.e. seed plus straw) are included. Due to the loss of leaves at maturity, the term apparent harvest index has been used to represent seed yield divided by above ground total dry matter production at maturity (Sernyk and Stefansson 1983). Apparent harvest index and harvest index have been shown to be positively correlated (r=0.97*) in soyabean (Schapaugh and Wilcox 1980).

Seed yield has been found to be positively correlated with growth characters. Thurling (1974 b) investigated the effect of sowing date on the seed yield and different morphological and growth characteristics of spring cultivars of oilseed rape species (B. rapa and B. napus and found that seed yield was correlated with total dry matter in B. napus (r=0.70*) and in B. rapa (r=0.42*) with harvest index. He also found that in B. napus, there was significant decline in seed yield with later sowing. The decline in seed yield was associated with a reduction in the total dry matter of the final harvest which in turn was most closely related to the duration of the vegetative phase of growth. Leaf area duration between anthesis and final harvest was found to be the most significant determinant of the total dry weight of the plants in B. rapa. Campbell and Kondra (1978) studied physiological characters in rapeseed, seed yield and its components using single plants of three B. napus cultivars and found that seed yield was significantly correlated with total dry matter per plant (r=0.93* to 0.95*) and the harvest index was also significantly correlated with yield per plant (r=0.21* to 0.52*). Yield improvement has always been a major objective of modern plant breeding and this improvement must be maintained to ensure that returns to farmers will be greater than the cost of production (Thurling 1991). The production of canola rapeseed hybrids and the utilization of the potential heterosis and particularly high parent heterosis is a practical approach to achieve seed yield improvement.

3. DIPLOTAXIS MURALIS (mur) CMS MAINTAINER OCCURRENCE AND FREQUENCY IN SUMMER RAPE (BRASSICA NAPUS L.)

3.1 ABSTRACT

Hand made crosses between a Diplotaxis muralis (mur) cytoplasmic male sterile (CMS) A-line and 101 summer rape (Brassica napus) genotypes from different sources (i.e. different countries) were produced in the greenhouse and evaluated in the field in an attempt to locate mur CMS maintainer genotypes in summer rape (B. napus), preferably those of canola quality.

The search for mur CMS maintainer genotypes revealed that there were no maintainer genotypes found in the summer rape (B.napus) genotypes evaluated, i.e. they were all restorers and, therefore, the frequency of occurrence of maintainer genotypes in summer rape is very low, possibly zero.

3.2 INTRODUCTION

Summer rape (Brassica napus) which belongs to the family Brassicaceae is an important oil crop in cool and humid agricultural areas. Improvements in seed quality (i.e. low erucic acid in the oil and low glucosinolates in the meal, Downey et al. 1975) producing "canola" quality summer rape and increased productivity have led to increased production of summer rape.

High levels of significant high-parent heterosis (20% to 70%) for seed yield have been obtained in hybrids of both spring and winter forms of oilseed rape (Olsson 1954, Schuster and Michael 1976, Hutcheson et al. 1981, Sernyk and Stefansson 1983, Grant and Beversdorf 1985, McVetty et al. 1989, Brandle and McVetty 1989, and Schuler et al.

1992). Significant heterosis has also been shown for other agronomic characters e.g. days to flowering, days to maturity and plant height (Sernyk and Stefansson 1983) and days to maturity, oil content and oil yield (Schuler et al. 1992)

The production of hybrids on a commercial scale in rapeseed that has small, hermaphrodite flowers which make hand pollination difficult require parental materials with an effective pollination control system. Several pollination control systems including self-incompatibility (SI)(Nasrallah et al. 1991), genetic male sterility (GMS)(Li et al. 1988), cytoplasmic male sterility (CMS) (Kaul 1988, McVetty, 1995) and male gametocides (Van der Meer and Van Dam 1979) have been developed or are under development. Among these, CMS is currently the most frequently used and the most extensively studied. Several male sterility inducing cytoplasms, which could potentially be developed into fully functional CMS systems include ogu, nap, oxy pol and mur systems. Nearly all the described systems have limitations that make their utilization in hybrid seed production difficult; for example, environmental (i.e. high temperature) sensitivity for the nap and pol CMS systems (Fan and Stefansson 1986, Burns et al. 1991) and high biological cost for the pol CMS system (McVetty et al. 1990, McVetty and Pinnisch 1994)

Diplotaxis male sterile cytoplasm and the derived CMS system (mur) in B. rapa was developed from interspecific crosses of D. muralis x B. rapa followed by transfer of the B. rapa genome into the cytoplasm of D. muralis by means of repeated back crosses (Hinata and Konno 1979). The male sterile plants were characterized by two nectaries, narrow petals and non dehiscent anthers which contained a small amount of pollen. Shiga

(1980) reported that the hybrids between D. muralis and B. napus (cv. Norin 16) were male sterile and he designated the male sterility as mur CMS. But Pellan-Delourme and Renard (1987) found that the crosses between cytoplasmic male sterile B. rapa with D. muralis cytoplasm and Norin 16 produced male fertile plants indicating that Norin 16 carried restorer genes for D. muralis cytoplasm. Restorer genes for the mur cytoplasm were found in B. rapa and in B. napus (Shiga 1980). Indeed all Canadian and European B. rapa cultivars tested fully restore male sterility induced by D. muralis cytoplasm (Fan et al. 1986) indicating the complete absence of maintainer genes in these cultivars. In spite of the fact that restorer genes for mur cytoplasm have been identified in B. napus, maintainer genes have not been found in summer cultivars of this species.

Attempts to obtain a mur CMS maintainer from summer type B. napus cultivar Regent were not successful (Fan et al. 1986). The mur cytoplasm was used to produce sterile plants in B. napus by means of repeated backcrosses; but following six back crosses of B. napus into mur cytoplasm and selecting for male sterility, it was found that the frequency of male steriles was low and all the male sterile plants carried an extra chromosome from D. muralis an indication of instability and hence the unreliability of the system.(Fan et al. 1986).

Pellan-Delourme and Renard (1987) investigating maintenance of mur CMS found that F1 plants obtained from crosses with B. napus cultivars Mangun and Hinchu were male sterile indicating that the two cultivars carried maintainer genes for this male sterility. The two cultivars are of Asiatic origin. Since many Asiatic B. napus cultivars are derived from crosses between B. napus and B. rapa (Shiga 1980), it is possible that

the presence of maintainers in Mangun and Hinchu result from this interspecific hybridization and not directly from B. napus. Preliminary tests have shown that Mangun is a winter habit rapeseed. The D. muralis CMS system has not been developed in summer rape (B. napus) due to a lack of maintainers.

The purpose of this study was to locate mur CMS maintainers in summer rape (B. napus), preferably in canola-quality genotypes, and then determine their frequency of occurrence for the male sterility inducing cytoplasm of D. muralis L.(DC)

3.3 MATERIALS AND METHODS

The experiments were conducted in the growth rooms, greenhouse and in the field on the University of Manitoba campus to investigate the maintenance of mur CMS inducing cytoplasm using summer oilseed rape (B. napus) genotypes as pollen parents. A cytoplasmic male sterile winter oilseed rape (B.napus) line (Mangun mur A and its maintainer (Mangun mur B) previously acquired by University of Manitoba were used as the CMS tester parents.

The male sterile line (Mangun mur A) was grown in the growth room in flats filled with metro-mix growth media. The seedlings were vernalized at 4^0C for 30 days to induce flowering. After vernalization, the seedlings were transplanted and grown in the greenhouse in 13 cm diameter pots (One seedling per pot) filled with a mixture of soil, sand and peat in a 2:1:1 ratio, respectively. One hundred and twenty plants Mangun mur A plants were grown to provide at least one pollen plant per cross.

One hundred and one summer rape B.napus genotypes, lines or cultivars from different sources (i.e. different countries) (Table 1) were also grown in the growth room and in the greenhouse and were used as pollen parents to make crosses with Mangun mur A at flowering. The photoperiod was 16 h and the temperatures were 20^0C by day and 17^0C by night for the growth room and 30^0C by day and 21^0C by night for the greenhouse. The photon flux density for the growth room was 340 uE m^{-2} s^{-1} and was provided by 1/3 GRO-LUX wide spectrum VHO lamps. The Mangun mur B-line was used as a control pollen parent. The F1's were grown in the field in 3 m single rows spaced 30 cm apart and male sterility and fertility were visually assessed at flowering based on observed absence or presence of pollen on the flower. At least 50 seeds per F1 were planted in the field. The data recorded was used to determine maintenance, or restoration of F1 of mur CMS plants.

3.4 RESULTS AND DISCUSSION

All F1 plants derived from all the crosses between the male sterile Mangun mur A-line and the 101 summer rape genotypes were fertile, whereas the F1 plants from the control cross were all sterile (Table 1 and 2) indicating that the 101 summer rape genotypes did not carry maintainer genes as expected but rather carried restorer gene(s) for mur CMS system. The results further indicated that the frequency of occurrence of maintainer genotypes in summer oilseed rape is very low, possibly zero.

Fan (1985) searched for mur cytoplasmic male sterility (CMS) maintainer genes in summer B. napus cultivar Regent. His attempts were not successful indicating that the

cultivar Regent does not carry maintainer genes for mur CMS. Shiga (1980) observed male sterile F1 plants from the cross between D. muralis and B. napus cv. Norin 16 but Pellan-Delourme and Renard (1987) crossed B. rapa with D. muralis cytoplasm to Norin 16 and obtained male fertile F1 plants indicating that Norin 16 carries restorer genes for the D. muralis cytoplasm. The difference in their observations could have been possibly due to use of different selections of Norlin 16. From the same experiments they found two cultivars 'Mangun' from South Korea and 'Hinchu' from Taiwan which carried maintainer genes for the mur CMS. They, however, indicated that the presence of the maintainer genes in the two cultivars results from the interspecific hybridization from crosses between B. rapa and B. napus from which many south Asian cultivars are derived. The results of this study showed absence of maintainer genes in B. napus genotypes tested and agrees with the postulation of Pellan-Delourme and Renard (1987) that the maintainer genes in Mangun and Hinchu originate from B. rapa and suggesting that the transfer of maintainer genes from Mangun mur B or some other source will facilitate the development of mur CMS system in summer oilseed rape.

3.5 CONCLUSIONS

An effective and utilizable cytoplasmic male sterility pollination control system is comprised of a male sterile A-line, a maintainer B-line and a restorer R-line. The search for maintainer genotypes in this study has revealed that there are no maintainer genotypes existing in the summer oilseed rape (B. napus) genotypes investigated and therefore, that the summer B. napus genotypes are restorers indicating that the frequency

of occurrence of maintainer genotypes is very low, possibly zero. The development of an utilizable mur CMS system in summer B. napus will require among other things, further search for maintainer genotypes or transfer of maintainer genes from the winter B. napus rapeseed cv. Mangun, or some other source into the summer B. napus materials.

Table 1. Summary of the country of origin, number of B.napus genotypes and phenotype of the hybrids between the B.napus genotypes and Mangun mur A

Country of Origin	Number of genotypes	F1 Phenotype
Australia	11	F*
Canada	28	F
China	8	F
Denmark	6	F
France	1	F
Germany	9	F
Holland	2	F
Japan	2	F
Pakistan	3	F
Poland	2	F
Sweden	18	F
Thailand	1	F
U.S.A	8	F
Miscellaneous	2	F
Total	101	F

* = Fertile

Table 2. Male fertility or sterility of F1 hybrids between B. napus genotypes and the CMS Mangun mur A-line

CUL/LINE	U of M NO.	COUNTRY	PLANTS TESTED	NO. STER	NO. FERT	PHTYP S/F
76n219	2431	Australia	24	0	24	F
Maluka	2410	Australia	30	0	30	F
73464rs	2165	Australia	110	0	110	F
Mamoo	2364	Australia	26	0	26	F
Rr22	2215	Australia	18	0	18	F
73-464	2322	Australia	12	0	12	F
Wesreo	2321	Australia	15	0	15	F
Wesbrook	2360	Australia	24	0	24	F
Tatyoon	2362	Australia	24	0	24	F
Bln 320	2449	Australia	27	0	27	F
Shiralee		Australia	15	0	15	F
Bln 320	2449	Canada	35	0	35	F
Stellar		Canada	78	0	78	F
Midas	2175	Canada	41	0	41	F
AC Excel		Canada	28	0	28	F
Ariel		Canada	34	0	34	F
Pivot		Canada	15	0	15	F
Altex	2447	Canada	29	0	29	F
Legend		Canada	26	0	26	F
Tower	2176	Canada	16	0	16	F
Vanguard		Canada	15	0	15	F
Regent	2025	Canada	31	0	31	F
Hero		Canada	33	0	33	F
Delta		Canada	28	0	28	F
365644	2150	Canada	12	0	12	F
Tobin	1433	Canada	24	0	24	F
Triton		Canada	27	0	27	F
	2334	Canada	27	0	27	F
Alto		Canada	24	0	24	F

Table 2. cont...

	NO.		TESTED	STER	FERT	S/F
Celebra		Canada	24	0	24	F
Bounty		Canada	24	0	24	F
Vanguard		Canada	24	0	24	F
Tristar		Canada	24	0	24	F
Ac 120		Canada	24	0	24	F
Hyola 401		Canada	24	0	24	F
Mercury		Canada	24	0	24	F
Cyclone		Canada	24	0	24	F
Profit		Canada	24	0	24	F
Westar		Canada	24	0	24	F
391553	2013	China	34	0	34	F
Chisaya	2315	China	30	0	30	F
Shang 23	2228	China	56	0	56	F
Pfk-79	2087	China	21	0	21	F
Erhao	2334	China	50	0	50	F
54731	2329	China	35	0	35	F
Sihao	2332	China	29	0	29	F
8 100 8	2346	China	32	0	32	F
Irego	2448	Denmark	24	0	24	F
Maribo-1-9015		Denmark	35	0	35	F
Maribo-1-9001		Denmark	27	0	27	F
Maribo-1-9026		Denmark	62	0	62	F
Maribo-1-9022		Denmark	29	0	29	F
Maribo-1-9007		Denmark	15	0	15	F
Brutor	2023	France	18	0	18	F
Goras	2052	Germany	10	0	10	F
S120365	2062	Germany	13	0	13	F
S120354	2055	Germany	42	0	42	F
Vanda	2001	Germany	19	0	19	F
Zollergo	2161	Germany	87	0	87	F
244/84	2405	Germany	32	0	32	F

Table 2. cont...

CULTIVAR/ LINE	U OF M NO.	COUNTRY	PLANTS TESTED	NO. STER	NO. FERT	PHTYP S/F
86/203	2407	Germany	21	0	21	F
S120365	2060	Germany	28	0	28	F
S120373	2064	Germany	25	0	25	F
75-03		Holland	52	0	52	F
Ischu 75-01		Holland	62	0	62	F
Ashai nat	2072	Japan	8	0	8	F
Michinoku	2317	Japan	19	0	19	F
251236	2006	Pakistan	34	0	34	F
K-72	2002	Pakistan	16	0	16	F
2511236		Pakistan	30	0	30	F
Bronowski	2027	Poland	19	0	19	F
311731	2084	Poland	21	0	12	F
305280	2131	Sweden	27	0	27	F
Havana	2357	Sweden	36	0	36	F
70-2810	2310	Sweden	42	0	42	F
70-5428	2264	Sweden	37	0	37	F
Sv 751504	2216	Sweden	16	0	16	F
Sv 71150	2289	Sweden	20	0	20	F
Global	2359	Sweden	26	0	26	F
71-2482	2247	Sweden	16	0	16	F
69-7862	2252	Sweden	39	0	39	F
Sv 7839801	2191	Sweden	25	0	25	F
Sv 763789	2218	Sweden	27	0	27	F
Sv 752543	2227	Sweden	23	0	23	F
Sv 705237	2261	Sweden	19	0	19	F
Topaz	2358	Sweden	27	0	27	F
Sv 691230	2254	Sweden	18	0	18	F
Sv 783901	2119	Sweden	36	0	36	F
Sv 705166	2250	Sweden	40	0	40	F

Table 2. cont...

CULTIVAR/ LINE	U OF M NO.	COUNTRY	PLANTS TESTED	NO. STER	NO. FERT	PHTYP S/F
Karat	2314	Sweden	24	0	24	F
	2151	Thailand	17	0	17	F
S11525	2382	USA	23	0	23	F
S1152-7	2383	USA	25	0	25	F
Kosa	2189	USA	36	0	36	F
Tira	2190	USA	15	0	15	F
Pol-b-1		USA	32	0	32	F
209303	2461	USA	30	0	30	F
31566	2373	USA	40	0	40	F
Orb 74183	2076	USA	34	0	34	F
	2448		34	0	34	F
Omega			11	0	11	F
Total	101	13				F
Control						
Mangun		N. Korea	70	70	0	S

F = Fertile
S = Sterile
Fert = Fertile
Ster = Sterile
PHTYP = Phenotype

4. INHERITANCE OF MAINTENANCE AND RESTORATION FOR THE DIPLOTAXIS MURALIS (L.) DC. (mur) CYTOPLASMIC MALE STERILITY SYSTEM IN SUMMER RAPE (BRASSICA NAPUS L.)

4.1 ABSTRACT

This study investigated the inheritance of Diplotaxis muralis (L) DC. (mur) cytoplasmic male sterile system (CMS) maintenance and restoration in summer rape (Brassica napus L.). Eight summer rape cultivars were crossed to a mur (CMS) A-line. The F1 progenies from all eight crosses were fertile and were selfed to produce the F2 generation. The F2 generation was grown in the field and data on segregation for fertility and sterility was recorded and analyzed. The F2 segregation data showed that mur CMS restoration in summer rape is controlled by one to three dominant genes and that cultivars differ in the number of genes they carry for the restoration of mur CMS. The ready availability of restorer genotypes suggests that the mur CMS system has good potential for hybrid seed production in summer rape, however, maintainer genotypes have first to be located or developed.

4.2 INTRODUCTION

Significant high-parent heterosis for seed yield (20 to 70%) have been obtained in hybrids of both spring and winter forms of oilseed rape (Olsson 1954, Schuster and Michael 1976, Hutcheson et al. 1981, 1983, Sernyk and Stefansson 1983, Grant and Beversdorf 1985, McVetty et al. 1989, Brandle and McVetty 1989, and Schuler et al. 1992). Significant mid-parent and high-parent heterosis have also been shown for quality

(Schuler et al. 1992). The discovery of significant levels of heterosis for seed yield in summer rape has created interest in the development of hybrids.

Production of hybrids requires an effective pollination control system to facilitate commercial hybrid seed production. One of the most widely used pollination control mechanism is cytoplasmic male sterility (CMS) system. Several male sterilizing cytoplasms, which could potentially be developed into fully functional CMS systems including ogu, nap, pol, nig, ctr and mur have been reported in the Brassica.

The ogu male sterile cytoplasm discovered by Ogura (1968) was developed by transferring the genome of B. napus into a male sterility inducing cytoplasm of radish (Raphanus sativus L.) (Bannerot et al. 1977). The initial ogu CMS materials were chlorotic at temperatures below 12^0 C, but this deficiency was corrected through protoplast fusion (Pelletier et al.1983, Delourme et al. 1991). The ogu CMS system should be fully functional after the current problems with restorer lines are overcome.

The nap male sterile cytoplasm was discovered in F2 generations from intercultivar crosses of several winter and spring cultivars with the cultivar Bronowski as the pollen parent (Shiga et al. 1983, Thompson 1972). The nap cytoplasm is the one in which most B. napus cultivars are found and, therefore the cytoplasm against which biological cost of all other potential CMS systems must be compared.

The pol male sterility system was discovered in the B. napus cultivar Polima (Fu 1981). The pol CMS system has potential for use in the production of hybrids but it has some limitations, including high temperature induced reversion to male fertility (Fan and Stefansson 1986, Burns et al. 1991) and high biological cost associated with this system

(McVetty et al. 1990 and McVetty and Pinnisch 1994). Thus a search for additional CMS system alternatives is necessary.

The Diplotaxis muralis male sterile cytoplasm and the derived (mur) CMS system in B. rapa was developed from D. muralis L.(DC)(Hinata and Konno 1979). Restorer genes for the mur cytoplasm were found in some B. rapa cultivars (Shiga 1980). All Canadian and European B. rapa cultivars tested restored male sterility induced by D. muralis cytoplasm (Fan et al. 1986). Pellan-Delourme and Renard (1987) investigating maintenance of mur CMS using 147 winter oilseed rape cultivars found that only F1 plants derived from crosses with two cultivars of winter rapeseed, Mangun and Hinchu, were male sterile indicating that these two cultivars carried maintainer genes for this male sterility while all the other genotypes were restorers for the mur cytoplasm. Though restorer genes for mur cytoplasm have been identified in winter B. napus rapeseed, their occurrence and inheritance in summer oilseed rape has not been investigated. The objective of this study was to investigate the inheritance and the number of genes involved in the restoration and maintenance of the male sterility inducing cytoplasm derived from D. muralis in B. napus.

4.3 MATERIALS AND METHODS

The experiments were conducted in the growth rooms, in the greenhouse and in the field on the University of Manitoba campus. A cytoplasmic male sterile line, Mangun mur A-line was acquired and used as the male sterile (CMS) test parent for making crosses with different summer oilseed rape cultivars. The cultivars used in this study were

Maluka and Marnoo from Australia, Ariel, Lergo and Karat from Europe and Regent, Stellar and Westar from Canada.

The male sterile line (Mangun mur A-line) was germinated in the growth room in flats filled with metro-mix growth media. The photoperiod was 16 h and the temperatures were 20^{0}C by day and 17^{0}C by night for the growth room and 30^{0}C by day and 21^{0}C by night for the greenhouse. The Photosynthetic photon flux density for the growth room was 340 uE m^{-2} s^{-1} and was provided by 1/3 GRO-LUX wide spectrum VHO lamps. At the 4th leaf stage, the seedlings were vernalized at 4^{0}C for 30 days to induce flowering. After vernalization, the seedlings were transplanted and grown in the greenhouse in 13 cm diameter fibre pots filled with soil, sand and peat in a 2:1:1 ratio, respectively. The eight summer rape cultivars were also grown in the growth room and in the greenhouse and were crossed to the male sterile line (Mangun mur A) plants at flowering.

The F1 was grown in the growth room and in the greenhouse. The F1 plants were selfed to generate F2 seed that was harvested from individual F1 plants and designated as families for each cross. The F2 was grown in the field at the Point; University of Manitoba campus. The plots consisted of single 3 m rows spaced 30 cm apart. At flowering, the F2 plants were visually assessed for sterility and fertility every two days. The assessed plants were counted and then cut and removed from the plots to avoid repeated counting of individual plants. The recorded data was analyzed using Chi-square procedures. Chi-square values for homogeneity of segregation ratios among families were calculated and found to be nonsignificant before the data for the families within each

cross were pooled. Chi-square goodness of fit tests were performed on the pooled data to determine the number of genes and the inheritance pattern.

4.4 RESULTS AND DISCUSSION

The F1 plants derived from all crosses were fertile (Table 1) indicating that all cultivars tested carried one or more dominant restorer genes for the mur CMS system. The segregation data from the F2 generation showed that 1 to 3 genes controlled the maintenance and restoration of male sterility for mur CMS and that the cultivars differ in the number of genes they carry for the maintenance and restoration (Table 2).

The data from the F2 generation derived from the cross Mangun mur A x Marnoo fit segregation ratios of 3:1 and 15:1 with non significant Chi-Square values of 2.53 and 0.71 respectively (Table 2), indicating that the cultivar Marnoo carried 1 or 2 genes for the restoration of mur CMS. The data from the F2 generation from the crosses of Mangun mur A with Maluka, Ariel, Regent, Westar and Stellar fit a segregation ratio of 15:1 with non-significant Chi-square values of 0.73, 2.80, 3.06, 1.88 and 2.90 respectively (Table 2), indicating that these cultivars carry two genes for the restoration of male fertility for mur CMS. The data from the F2 generation from the crosses Mangun mur A x Lergo and Mangun mur A x Karat fit segregation ratios of 15:1 and 63:1 with Chi-square values of 3.49 and 1.07 for Lergo and 3.06 and 2.65 for Karat respectively (Table 2), indicating that Lergo and Karat carry two or three genes for restoration of mur CMS. The segregation ratios indicated that maintenance is recessively inherited while restoration is dominantly inherited. The variation in the number of restorer genes within cultivars is a reflection of

their heterogeneity (they are all open pollinated cultivars), whereas the variation in number of restorer genes among cultivars is due to differences in genetic constitution of the cultivars from different geographical locations.

CMS restoration is by nuclear genes, frequently dominant in action, and in many cases few in number, i.e. one to four dominant genes sometimes with minor male fertility modifiers to maintain complete restoration in some environments (McVetty et al. 1995). The cytoplasmic male sterility (CMS) restorer genes suppress the expression of CMS permitting normal or near normal pollen production. In summer rape, restorer genes for the other important CMS systems (ogu, nap and pol) have been identified and this, in addition to availability of maintainers, has attracted interest in hybrid seed production. Fan (1985) reported that the nap CMS requires one dominant gene "Rf1" for restoration while the ogu CMS requires two dominant genes "*Rf1*" and "*Rf2*" both derived from European radish (Heyn 1978). Male fertility restoration was accompanied by reduction in female fertility (Pellan- Delourme and Renard 1988). The problem of impairment of female fertility has not yet been solved and will have to be overcome before the ogu CMS system becomes fully functional (Renard 1995). Oilseed rape with the pol CMS requires one restorer gene *Rf1* from the winter cultivar Italy or a different single restorer gene from UM2353, for fertility restoration (Fang and McVetty 1989). However, the availability of only a few stable maintainers and high temperature induced reversion to male fertility limit the versatility of the pol CMS system for hybrid seed production (Sodhi et al. 1993). In B. rapa carrying Diplotaxis muralis (mur) cytoplasm, some B. rapa varieties were found to have restorer genes for the mur CMS system and the varieties or

the substitution lines were designated as (mur) *Rfm Rfm* and (mur) *Rfc Rfc rfm rfm*, signifying two dominant genes for the restoration the mur CMS (Shiga 1980).

In this study, it has been shown that all eight summer rape cultivars carry restorer genes for the restoration of mur CMS and that the cultivars differ in the number of genes they carry. This study also agrees with the findings of Pellan-Delourme and Renard (1987) who found that of the 147 winter rape cultivars evaluated for maintenance of mur CMS, only two lines maintained the sterility and all the other genotypes carried restorer genes. This study, as does that of Pellan- Delourme and Renard (1987) suggests that there are numerous restorer genotypes for the mur CMS system and this availability should permit, as with ogu and pol, permit large scale hybrid seed production using mur CMS, however the search for mur CMS maintainer genotypes should be continued to broaden the maintainer genetic base.

4.5 CONCLUSIONS

The production and utilization of hybrids involves the development of inbred lines or parents with an effective pollination control system to permit commercial hybrid seed production. One of the most studied pollination control systems is the cytoplasmic male sterility system that is comprised of a male sterile A-line, a maintainer B-line and a restorer R-line. Our study of restoration and maintenance of mur CMS system demonstrated that restoration and maintenance of mur CMS system is controlled by one to three genes and that the cultivars tested differed in the number of genes they carry for the restoration and maintenance. All of the cultivars tested restored the male fertility

suggesting that all or most of the summer rape cultivars are restorers of fertility for the mur CMS system. This ready availability of restorers should permit the development and utilization of mur CMS in summer rape, once stable maintainers have been located or developed.

Table 1. Country of origin and phenotype of the F1 generation of crosses between Mangun mur A-line and eight summer rape cultivars

Country of origin	Cultivar	F1 phenotype
Australia	Maluka	Fertile
Australia	Marnoo	Fertile
Denmark	Ariel	Fertile
Denmark	Lergo	Fertile
Sweden	Karat	Fertile
Canada	Regent	Fertile
Canada	Stellar	Fertile
Canada	Westar	Fertile

Table 2. Male fertility:sterility segregation ratios and X^2 tests for the F2 generation of crosses between Mangun mur A-line and eight summer rape cultivars

Cross	Total no. of plants	Observed segregation Fertile	Sterile	Expected ratio	X^2	P
Mangun mur A x Maluka	1904	1794	110	15:1	0.73	0.30-0.50
Mangun mur A x Lergo	592	566	26	15:1	3.49	0.05-0.10
	897	883	14	63:1	0.00	0.95-0.99
Mangun mur A x Marnoo	629	489	140	3:1	2.53	0.10-0.20
	898	848	50	15:1	0.71	0.30-0.50
Mangun mur A x Karat	1912	1811	101	15:1	3.06	0.05-0.10
	734	728	6	63:1	2.65	0.10-0.20
Mangun mur A x Ariel	1251	1187	64	15:1	2.80	0.05-0.10
Mangun mur A x Regent	1964	1860	104	15:1	3.06	0.05-0.10
Mangun mur A x Westar	2184	2063	121	15:1	1.88	0.10-0.20
Mangun mur A x Stellar	831	767	64	15:1	2.90	0.05-0.10

5. DEVELOPMENT AND EVALUATION OF DIPLOTAXIS MURALIS (L) DC (mur) CYTOPLASMIC MALE STERILITY (CMS) SYSTEM IN SUMMER RAPE (BRASSICA NAPUS L.)

5.1 ABSTRACT

The Diplotaxis muralis male sterility inducing cytoplasm and its maintainer genes were transferred from the winter rapeseed (B. napus) male sterile Mangun mur A-line and maintainer Mangun mur B-line carrying the sterile cytoplasm and maintainer genes, respectively, into the summer rape cultivars Regent and Karat through a backcross approach using test crosses in each cycle of back crossing. Three pairs of summer rape mur CMS A-lines and B-lines, Regent 14, Karat 7-5 and Karat 7-14 were developed. Seedling emergence, days to first flower, days to maturity and height of the newly developed summer rape mur CMS A-lines and B-lines were compared. The high temperature stability of the newly developed mur CMS A-lines was also evaluated. The newly developed mur CMS A-lines and B-lines were generally equal in emergence, flowering, maturity and height. The A-line and B-line pairs were on average 5 days later to first flower and 3 days later to maturity than their respective open pollinated summer rape cultivars. The male sterility of the newly developed mur CMS A-lines was stable at temperatures of 30/24^0 C.

5.2 INTRODUCTION

Observations of high levels of high parent-heterosis for seed yield (20% to 70%) in hybrids of both spring and winter forms of rapeseed (Hutcheson et al. 1981, Sernyk

and Stefansson 1983, Grant and Beversdorf 1985, Brandle and McVetty 1989 McVetty et al.1990, Schuler et al. 1992) have created interest in the development and utilization of hybrids in rapeseed. Economical production of hybrid seed on a commercial scale requires an effective pollination control system. The pollination control systems that have been developed or are under development for use in hybrid development include hand emasculation and pollination (Poehlman 1979), self-incompatibility (SI)(Nasrallah et al. 1991), genetic male sterility (GMS)(Li et al. 1988), cytoplasmic male sterility (CMS) (Kaul 1988, McVetty, 1995) and male gametocides (Van der Meer and Van Dam 1979). Among these, CMS is currently the most frequently used and the most extensively studied. Several pollination control systems including self-incompatibility (SI), genetic male sterility (GMS), cytoplasmic male sterility (CMS) male gametocides and recently, genetically-engineered male sterility have been developed or are under development. Of these, CMS is the most widely used and most commonly studied pollination control mechanism.

Male sterilizing cytoplasms, which could potentially be developed into fully functional CMS systems in Brassica include nap, ogu, pol and mur. The ogu male sterile cytoplasm discovered by Ogura (1968) was developed by transferring the genome of B. napus L. into a male sterility inducing cytoplasm of radish (Raphanus sativus L).(Bannerot et al. 1977). The utilization of ogu cytoplasm was initially limited by: chlorosis, expressed at temperatures below 12^0 C, for plants carrying the ogu cytoplasm; the impairment of female fertility and lack of restorer genes. These deficiencies were

overcome through protoplast fusion and transfer of restorer genes from Raphanus sativus (Pelletier et al. 1983, Pelletier 1990, Delourme et al. 1991) making the ogu CMS system potentially functional (Renard et al. 1992).

The nap male sterile cytoplasm was discovered in F2 generations from intercultivar crosses of several winter and spring rape cultivars with the cultivar Bronowski as the pollen parent (Shiga et al. 1983, Thompson 1972). The pol male sterile cytoplasm was discovered in the winter rape cultivar Polima (Fu 1981). The nap and pol CMS systems are limited by high temperature induced male fertility reversion (Fan et al. 1986, Fu et al. 1990, Burns et al. 1991). Hybrids in the pol cytoplasm also yield less (seed yield was reduced by 23%) and have reduced oil content (relative oil content was reduced by 3%) than genetically identical hybrids in the nap cytoplasm (McVetty et al. 1990). McVetty and Pinnisch (1994) also evaluated the effect of nap and pol cytoplasm on the performance of three pairs of summer rape isolines and found that one nap line yielded significantly more seed (17% more) than the corresponding pol line, while three nap isolines had significantly higher protein content (2 to 3% more) than their corresponding pol lines. These results indicate that there are pleiotropic negative effects (i.e. a biological cost) associated with the pol cytoplasm.

Though the ogu CMS system may eventually be found to be fully functional, there is still need to diversify the cytoplasms by developing additional CMS systems in summer rape, not only to expedite commercial hybrid seed production, but also as a safeguard against disease susceptibility and lack of disease resistance of some cytoplasms, for

example southern leaf blight susceptibility associated with the T-cytoplasm of corn (Ullstrup 1972).

One of the potential CMS systems under development is the mur CMS system. Diplotaxis muralis male sterile cytoplasm and the derived CMS system (mur) in B. rapa was developed from D. muralis (L.) D.C. (Hinata and Konno (1979). Restorer and maintainer genes for mur cytoplasm have been identified in winter rapeseed, (Pellan-Delourme and Renard 1987), but they have not been developed and evaluated in summer rape cultivars. Attempts by Fan et al. (1986) to obtain a mur CMS maintainer line from the summer rape cultivar Regent were not successful, because after repeated backcross to D. muralis using the cultivar Regent as a recurrent parent, the frequency of male sterile plants was low and this frequency dropped with each successive back cross.

There is, therefore, the need for identification of maintainer genotypes in summer rape or to transfer maintainer genes from B. rapa or some other source (e.g. Mangun mur B) into summer rape to facilitate the development of a D. muralis based CMS. A comparison of phenological and agronomic traits of the newly developed mur CMS A-lines and B-lines could reveal any biological (negative or positive effects) association with the mur cytoplasm. The high temperature instability of the nap and pol CMS A-lines suggests that it would be prudent to check the temperature stability of the mur CMS A-lines.

The purposes of this study were; 1. to develop mur CMS A-lines and B-lines in summer rape, 2. to study the effects of the mur cytoplasm on the phenology and

agronomic performance of these lines and 3. to investigate the effect of high temperature treatment on the expression of male sterility in summer rape mur CMS A-lines.

5.3 MATERIALS AND METHODS

5.3.1 *Development of mur CMS A-lines and B-lines in summer rape*

The development of mur CMS A-lines and B-lines was carried out in the greenhouse at the University of Manitoba campus from 1990 to 1993. The winter rapeseed lines Mangun mur A and Mangun mur B, carrying the male sterile cytoplasm and maintainer genes, respectively, for mur CMS were used as the sources for D. muralis male sterility inducing cytoplasm and maintainer genes. The open pollinated summer rape cultivars Karat, Marnoo and Regent provided the summer rape genetic backgrounds used in this study.

The development of the mur CMS A-lines and B-lines was conducted in three stages as follows:

1. The transfer of maintainer genes from the Mangun mur B-line to selected summer rape genetic backgrounds to develop mur CMS summer rape B-lines (Figure 1).
2. The use of test crosses using the Mangun mur A-line as the female test parent, to facilitate the selection of maintainer genotypes from the F2 generation of the [Mangun mur B-line X selected summer rape cultivars] developed in (1) above (Figure 1).

3. The use of a back cross series, using the selected summer rape mur CMS B-lines developed in (2) above, as the recurrent parents in crosses with maintained and selected F1's from the crosses [Mangun mur A x (Mangun mur B X selected summer rape cultivar F2's)], to transfer the nuclear genes of the selected summer rape mur B-lines into the sterile cytoplasm from D. muralis (Figure 1).

5.3.1.1. *Transfer of maintainer genes.*

The Mangun mur B-line was grown in a growth room in flats filled with metro-mix growth media for 21 days and then the seedlings were transplanted into 13 cm diameter fibre pots filled with soil, sand and peat in the ratio 2:1:1. The transplants were then vernalized for 30 days at 4^0 C in a cold room before being placed in the greenhouse. At flowering, Mangun mur B plants were crossed as to the summer rape cultivars Karat, Marnoo and Regent to transfer the mur CMS maintainer genes to these summer rape genetic backgrounds. The F1 plants were grown in the greenhouse and were selfed to generate F2 seed that was harvested separately from each F1 plant and later utilized in test crosses. The F2 plants which were test crossed were also selfed to produce an F3 generation that was later used in the back cross series.

5.3.1.2 *Test crosses to select maintainers and maintained F1's*

Twenty-nine F2 plants from each of the [Mangun mur B X selected summer rape cultivars] crosses were grown in the greenhouse and used to pollinate vernalized plants of the Mangun mur A-line. Ten F1 seeds for each test cross were grown in the

greenhouse and the plants that developed to flowering stage were assessed for expression of male sterility and fertility. The F1 groups were of three types: uniformly male sterile, uniformly male fertile and segregating for male sterility and fertility. The uniformly male sterile F1 groups indicated that the mur cytoplasm induced male sterility had been maintained and, therefore, that the F2 plant used as pollen parent carried mur CMS maintainer genes in the homozygous condition. Conversely, uniformly male fertile F1 groups indicated that the F2 plant used in the test cross possessed one or more restorer genes in the homozygous state. The F1 groups which segregated for male sterility and fertility indicated that the F2 plant used in the test cross was heterozygous for all mur CMS maintainer/restorer genes. Twenty-nine F3 plants derived from [Mangun mur B x Karat F2's] were subsequently test crossed to the vernalized Mangun mur A-line. [Mangun mur B x Regent F3] plants and [Mangun mur B x Marnoo F3] plants were also test crossed to the vernalized Mangun mur A-line to confirm the ability of these genotypes to maintain the mur CMS male sterility.

The test crosses of the F3 plants were grown in the greenhouse. Twenty-four F1 plants from each F3 plant test crossed were assessed for male sterility and fertility.

To expedite development of the mur CMS A-lines in summer rape, the maintained F1's from the Mangun mur A x (Mangun mur B x Karat F3), Marnoo mur A x (Mangun mur B x Marnoo F3) and Marnoo mur A x (Mangun mur B x Regent F3) test crosses were selected and crossed with their respective F3 maintainer genotypes. Crosses with the Marnoo maintainer line were discontinued during the development program due to

difficulties in synchronizing the flowering between Mangun mur A and the Marnoo F3 maintainer genotype plants.

5.3.1.3 *Backcross series*

The F1's of the [Mangun mur A x Mangun mur B x Karat F3], Mangun mur A x Mangun mur B x Marnoo F3) and Mangun mur A x (Mangun mur B x Regent F3) which were male sterile (i.e. maintained F1 plants), were crossed with their respective selected maintainer F3 plants that were grown from reserve seed simultaneously as the test cross was underway to produce a back cross (BC1F1) generation. The back cross series was repeated up to BC6F1 using a test cross in each cycle of back crossing. The back crossing led to the development of three mur CMS male sterile lines and associated maintainer lines. The phenological and agronomic characteristics of these mur A-lines and mur B-lines were compared to each other and with their originating summer rape parents. These studies were conducted in the greenhouse.

5.3.2 *Evaluation of mur CMS A-lines and B-lines in summer rape*

5.3.2.1 *Floral morphology comparisons*

A comparative flower morphology study was undertaken on the summer rape mur CMS A-lines and mur CMS B-lines for plants grown in the greenhouse. These plants were grown as described in the phenological and agronomic assessment comparisons study detailed below.

5.3.2.2 *Phenological and agronomic characteristic comparisons*

The phenological and agronomic characteristics of the newly developed summer rape mur CMS A-lines and mur CMS B-lines were compared to each other. The mur CMS A-lines and mur CMS B-lines were also compared with their originating summer rape parents.

The Mangun mur A-line and mur B-line were grown in growth room for 21 days in flats filled with metro-mix growth media and then the seedlings were transplanted into 13 cm diameter fibre pots filled with soil, sand and peat in the ratio 2:1:1. and placed in the greenhouse.

Figure 1. Flow chart for development of Diplotaxis muralis CMS system A-lines and B-lines in summer rape (Brassica napus L.)

Step 1. Summer rape cultivar mur B-line development

MANGUN mur B X (summer rape cultivars)
|
F1
| (self)
F2 mur B
| (self)
F3 mur B families

Step 2. Test crosses to identify F2 or F3 mur B genotypes

MANGUN mur A X (summer rape cultivars) F2 mur B
(29 F2 plants per cultivar test crossed)
|
F1 (10 plants grown from each test crossed F2 plant)

SELECT (MANGUN mur A x [summer rape cultivars] F2 mur B) plants and [summer rape cultivars] F2 mur B plants

MANGUN mur A X (summer rape cultivars) F3 mur B
|
F1 (24 plants grown from each test crossed F3 plant)

SELECT (MANGUN mur A X [summer rape cultivars] F3 mur B) plants and [summer rape cultivars] F3 mur B plants.

Step 3. Back crosses to develop summer rape cultivar mur A-lines and mur B-lines

[MANGUN mur A x (F3 mur B)] X [summer rape cultivars](F3 mur B) BC1

[MANGUN mur A x (F3 mur B)] X [summer rape cultivars](F3 mur B) BC2

[MANGUN mur A x (F3 mur B)] X [summer rape cultivars](F3 mur B) BC3

[MANGUN mur A x (F3 mur B)] X [summer rape cultivars](F3 mur B) BC4

[MANGUN mur A x (F3 mur B)] X [summer rape cultivars](F3 mur B) BC5

[MANGUN mur A x (F3 mur B)] X [summer rape cultivars](F3 mur B) BC6

[summer rape cultivars] mur A-lines [summer rape cultivars] mur B-lines

The transplants were then vernalized for 30 days at 4^0 C in a cold room before being placed in the greenhouse. Mangun mur A-line and Mangun mur B-line plants, Karat mur A-line and mur B-line plants, and Regent mur A-line and mur B line plants were grown in a growth room for 21 days in flats filled with metro-mix growth media and then the seedlings were transplanted into 13 cm diameter fibre pots filled with soil, sand and peat in the ratio 2:1:1, before being placed in the greenhouse. The lines studied were assessed for days to emergence, days to first flower, days to maturity and height.

5.3.3 *High temperature stability of summer rape mur CMS A-lines*

A vernalized Mangun mur A-line, two Karat mur A-lines, Regent mur A, and a control CMS A-line, Karat pol A, (the best available pol CMS A-line), were grown in temperature in a growth room and temperature controlled growth cabinet.

Thirty six seeds of each male sterile line were germinated in flats filled with metro-mix growth media and placed in a $22/16^0$ C growth room with a 16 hr photoperiod and watered daily. After 3 weeks, the seedlings were transplanted into 13 cm diameter fibre pots filled with soil, sand and peat (2:1:1). The Mangun mur A-line was vernalized for 30 days at 4^0 C. All of the male sterile line plants were placed in a growth room and grown at approximate temperatures of $22/16^0$ C (day/night). The photoperiod was 16 h. The Photosynthetic photon flux density was 340 uE m-2 s^{-1} and was provided by 1/3 GRO-LUX wide spectrum VHO lamps. The design used was a completely randomized design with six replicates.

At growth stage 3.1 (budding stage)(Harper and Berenkamp 1975), eighteen of the plants of each entry were transferred to a growth cabinet (Burns et al. 1991). The photoperiod was 16 h and the temperatures were 3^0C by day and 24^0C by night for the growth room. The Photosynthetic photon flux density for the growth cabinet was 396 *u*E m^{-2} s^{-1} and was provided by High Pressure Na lamps. After seven days of high-temperature treatment, the high-temperature treated plants were returned to the growth room set at $22/16^0$ C.

All the plants grown under cool and high temperature treatment conditions were assessed for fertility reversion starting 14 days following the end of the high temperature treatment. The assessment was conducted every two days for a period of 3 weeks or until flowering was completed. To ensure consistent flower age during assessment, the third open flower from the top of the raceme was always removed and scored for fertility. Sepals and petals were removed and the number of anthers that were dehiscent or possessing swollen pollen filled locule(s) were counted. A magnifying glass was used to facilitate more accurate assessment. Each flower was rated on a male sterility index between 0 and 6 with 0 indicating none of the anthers with pollen and 6 indicating a flower with all the 6 anthers that produced pollen (Burns et al. 1991). The mean rating of each entry in a treatment was calculated over replications and days. Analysis of variance was performed on the data and mean comparisons were determined using Duncan's Multiple Range Test at 0.05 probability.

5.4 RESULTS AND DISCUSSION

5.4.1 *Development of mur CMS A-lines and B-lines in summer rape*

Test cross results indicated that one F1 group derived from the crosses between Mangun mur A X (Mangun mur B X Marnoo F2's) plant 21, and one F1 group derived from the cross between and Mangun mur A X (Mangun mur B X Regent F2's) plant 14, consisted of male sterile plants only, indicating that these two F2 plants were homozygous mur CMS maintainer genotypes (Tables 1 and 2). In contrast, no F1 groups derived from the cross Mangun mur A X (Mangun mur B X Karat F2's) consisted of exclusively male sterile plants (Table 3). Several F1 groups displayed segregation for male fertility and sterility in this set of test crosses, indicating that the F2 plants were heterozygous for mur CMS maintainer genes. However, test crosses of the (Mangun mur B X Karat 7-5 F3) plants and the (Mangun mur B X Karat 7-14 F3) plants produced two F1 groups that consisted entirely of male sterile plants, indicating that these F3 plants were homozygous for mur CMS maintainer genes (Table 4). The F3 generation plants of Regent 14, Karat 7-5 and Karat 7-14 were used as the recurrent maintainer genotype (B-line) parents in a backcross series and that led to development of 3 mur CMS A-line and B-line pairs. The approach was slightly different from those used in the development of ogu (Bannerot et al. 1977) or nap (Shiga and Baba 1971, 1973) A-lines and B-lines where A-line development was accomplished directly from maintainer genotypes. In this study, the maintainer genes had first to be transferred from a source genotype (Mangun mur B) to summer rape genetic backgrounds before those lines with maintainer genes could be used

as the recurrent parents to develop mur CMS A-lines in summer rape. The developed mur CMS A-lines are completely sterile while their maintainer lines are fully fertile (Figures 2 and 3), showing that it is possible to develop fully acceptable mur CMS A-lines and B-lines in summer rape.

Table 1. F1 progeny phenotype for the test cross of Mangun mur A X (Mangun mur B X Marnoo F2)

Test cross group number[+]	F1 progeny phenotype		
	Sterile	Fertile	Partially Sterile
1	0	8	0
2	0	7	0
3	1	2	2
4	0	7	0
5	0	9	0
6	0	5	3
7	0	8	1
8	1	6	3
9	0	7	0
10	0	10	0
11	0	8	0
12	3	6	0
13	0	9	0
14	0	8	0
15	0	7	0
16	0	9	0
17	0	8	0
18	0	6	0
19	0	5	0
20	0	8	0
21	8	0	0
22	1	3	2
23	0	9	0
24	0	5	0
25	0	7	2
26	0	9	0
27	0	8	0
28	0	5	1
29	0	6	0

[+] also = F2 plant number

Table 2. F1 progeny phenotype for the test cross of Mangun mur A X (Mangun mur B X Regent F2)

Test cross group number+	F1 progeny phenotype		
	Sterile	Fertile	Partially Sterile
1	5	3	0
2	0	8	0
3	7	0	2
4	5	2	0
5	0	9	0
6	1	9	0
7	0	8	1
8	3	3	1
9	2	5	1
10	2	4	3
11	2	6	0
12	2	5	1
13	3	4	1
14	10	0	0
15	0	7	1
16	6	0	3
17	1	8	1
18	0	6	0
19	4	6	0
20	1	8	1
21	1	9	1
22	7	0	0
23	7	1	1
24	0	5	0
25	1	7	2
26	0	9	1
27	7	3	1
28	2	2	1
29	1	1	3

+ also = F2 plant number

Table 3. F1 progeny phenotype for the test cross of Mangun mur A X (Mangun mur B X Karat F2)

Test cross group number+	F1 progeny phenotype		
	Sterile	Fertile	Partially Sterile
1	0	10	0
2	0	8	0
3	1	6	2
4	0	10	0
5	0	9	0
6	0	5	0
7	1	3	2
8	0	1	8
9	0	7	1
10	1	7	0
11	0	6	0
12	1	3	1
13	0	9	0
14	2	4	3
15	0	7	0
16	0	6	0
17	0	8	0
18	0	6	0
19	0	8	0
20	0	0	7
21	0	4	1
22	0	6	2
23	0	8	0
24	0	5	0
25	0	7	0
26	0	9	0
27	0	8	0
28	0	7	0
29	1	2	2

+ also = F2 plant number

Table 4. F1 progeny phenotype for the test cross of Mangun mur A X (Mangun mur B X Karat 7-5 F3), Mangun mur A X (Mangun mur B X Karat 7-14 F3), Mangun mur A X (Mangun mur B X Marnoo 21 F3) and Mangun mur A X (Mangun mur B X Regent 14 F3)

Test cross group number+	F1 progeny phenotype		
	Sterile	Fertile	Partially Sterile
K 7-5	24	0	0
K 7-14	24	0	0
M 21	24	0	0
R 14	24	0	0

+ also = F3 plant number

5.4.2 *Evaluation of mur CMS A-lines and B-lines in summer rape*

5.4.2.1 *Floral morphology comparisons*

Mangun mur A plants have flowers with "normal" (fully developed with no malformation of the floral parts) sepals and style, smaller and pointed petals, shorter filaments and anthers without pollen and occasional petaloidy compared to Mangun in the nap (mur B) cytoplasm (Figure 2C, 2D). The Mangun mur B plants have completely "normal" flowers (Figure 2A, 2B).

Regent mur A plants are characterized by flowers with larger and malformed sepals and style, smaller and rounded petals, shorter and occasionally absent anthers

without pollen and occasionally petaloidy compared to Regent in the nap (mur B) cytoplasm (Figure 3C, 3D). The Regent mur B plants have completely normal flowers (Figure 3A, 3B).

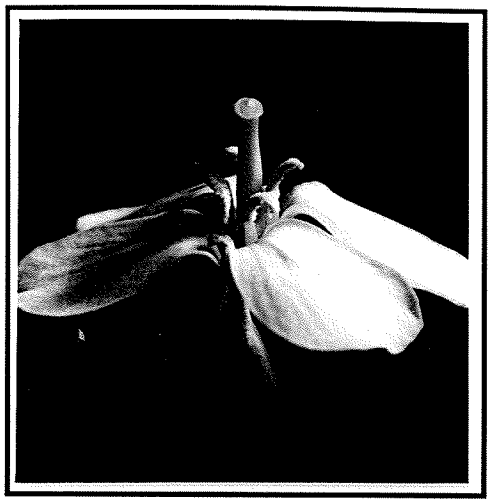
Karat mur A plants have flowers with normal sepals and style, smaller and pointed petals, shorter filaments and anthers without pollen and occasionally petaloidy compared to Karat in the nap (mur B) cytoplasm (Figure 4C, 4D). The Karat mur B plants have completely normal flowers (Figure 4A 4B).

Figure 2 A. Mangun mur B flower.

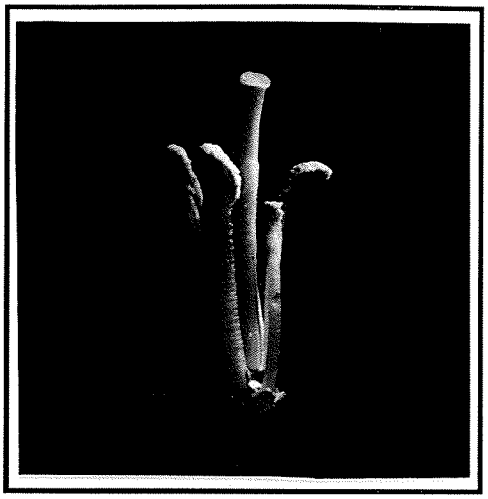
Figure 2 B. Mangun mur B flower with sepals and petals removed.

Figure 2 C. Mangun mur A flower with sepals and petals.

Figure 2 D. Mangun mur A flower with sepals and petals removed.



A



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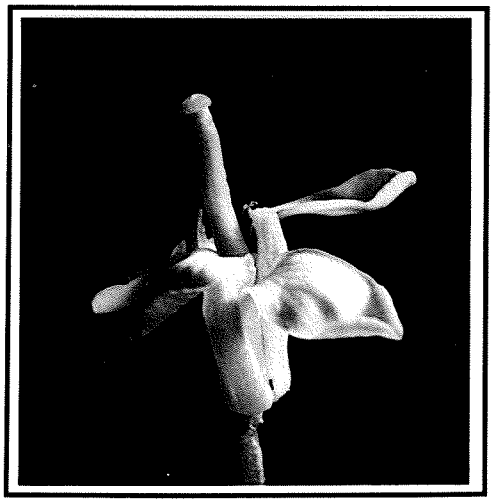


C

D

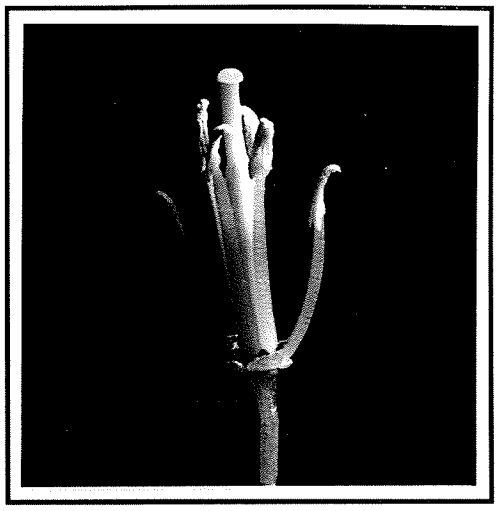
Figure 3 A. Regent mur B flower.

Figure 3 B. Regent mur B flower with sepals and petals removed.

Figure 3 C. Regent mur A flower with sepals and petals.

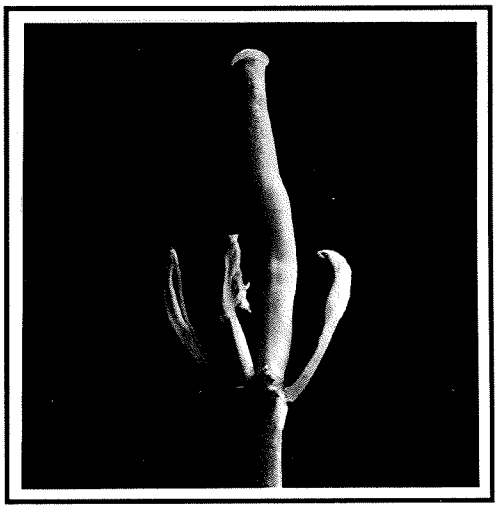
Figure 3 D. Regent mur A flower with sepals and petals removed.

A



B

C



D

Figure 4 A. Karat mur B flower.

Figure 4 B. Karat mur B flower with sepals and petals removed.

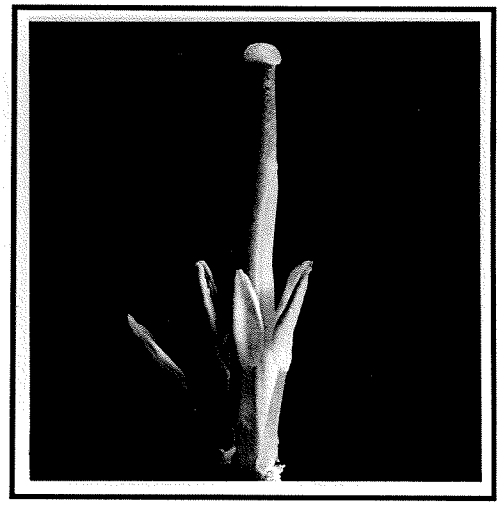
Figure 4 C. Karat mur A flower with sepals and petals.

Figure 4 D. Karat mur A flower with sepals and petals removed.

A

B

C



D

5.4.2.2 *Phenological and agronomic characteristic comparisons*

The Mangun mur A-line and Mangun mur B-line emerged one day later than all other lines and open pollinated populations (Table 5). This may be simply a reflection of genotype related seed vigour differences. More importantly, the mur A-lines for all cultivars were not different in days to emergence from their respective mur B-lines. There is therefore, no apparent "detrimental" effect of the mur cytoplasm on days to emergence.

The unvernalized Mangun mur A-line and mur B-line were significantly later in days to first flower than all other lines and open pollinated populations, including vernalized Mangun mur A-line and mur B-line (Table 5). The Mangun response to vernalization confirms the presumed winter habit of this cultivar. Four pairs of mur A-lines and mur B-lines were not different in days to first flower while two pairs of mur A-lines and mur B-lines were significantly different in days to first flower, with the mur A-lines flowering later than their respective mur B-lines. These results suggest that there may some detrimental effect of the mur cytoplasm on days to first flower, at least for some genotypes. In general, the Karat and Regent mur A-lines and mur B-lines were later in days to first flower than their respective open pollinated populations, a reflection of the Mangun genes for later flowering that are apparently still present in these mur A-lines and mur B-lines.

The unvernalized Mangun mur A-line and mur B-line were significantly later in days to maturity than all other lines and open pollinated populations, including vernalized

Mangun mur A-line and mur B-line (Table 5). The Mangun response to vernalization confirms the semi-winter habit of this cultivar. Five pairs of mur A-lines and mur B-lines were not different in days to maturity while one pair of mur A-line and mur B-line were significantly different in days to maturity, with the mur A-lines maturing later than their respective mur B-lines. These results suggest that there may be some "detrimental" effect of the mur cytoplasm on days to maturity, at least for some genotypes. In general, the Karat and Regent mur A-lines and mur B-lines were not different in days to maturity from their respective open pollinated populations.

The unvernalized Mangun mur A-line was taller than all other lines and open pollinated populations, including unvernalized Mangun mur B-line (Table 5). The remaining five pairs of mur A-lines and mur B-lines were not different in height. There would appear to be little detrimental effect of the mur cytoplasm on height. The Regent mur A-line and mur B-line were not different in height from the Regent open pollinated population, while the Karat mur A-line and mur B-line were significantly taller than the Karat open pollinated population. Again, there is no evidence to suggest that there is any detrimental effect of the mur cytoplasm on height.

Table 5. Days to emergence, days to first flowering, days to maturity and height for mur CMS A-lines and B-lines and selected open pollinated population summer rape cultivars

TREATMENT	Emergence (Days)	First Flowering (Days)	Maturity (Days)	Height (cm)
Mangun mur A	5a	77a	127a	130a
Mangun mur B	5a	73b	119b	115b
Mangun mur A(V)	5a	48g	89cd	112b
Mangun mur B(V)	5a	47g	88d	112b
Regent mur A	4b	55cd	92cd	113b
Regent mur B	4b	52def	87d	112b
Karat 14 mur A	4b	59c	92cd	115b
Karat 14 mur B	4b	48g	87d	115b
Karat 5 mur A	4b	55cd	94c	115b
Karat 5 mur B	4b	53de	89cd	115b
Regent (opp)	4b	49fg	89cd	111b
Karat (opp)	4b	51efg	92cd	95c
GRAND MEAN	4.5	56	95	113
C.V. (%)	11.0	17.4	13.7	7.1

Means in a column followed by the same letter are not significantly different as determined by Duncan's Multiple
Range Test at 0.05 probability level.
V = Vernalized

5.4.3 *High temperature stability of summer rape mur CMS A-lines*

Exposure of the mur CMS A-lines and the pol CMS A-line for 7 days to day/night temperatures of 30/24^0 C in a controlled environment cabinet did not lead to reversion to male fertility in any of the mur CMS A-lines, but it did lead to male fertility reversion in the pol CMS line (Table 6). The mur CMS A-lines are temperature stable, at least to 30/24^0 C. Male sterility index values of 0 were recorded for all replicates of all mur CMS A-lines in both 22/16 ^{0} C and 30/24 ^{0} C temperature regimes. Male sterility index values of 0 were recorded on the Karat pol CMS A-line used as the control at 22/16^0 C, showing that at low temperature, male sterility was not affected. However, a mean male sterility index of 2.7 was recorded for the Karat pol CMS A-line when it was high-temperature treated (Table 6).

These results on pol A sensitivity are in agreement with those of Fan et al. (1986) and Burns et al. (1991). Fan et al. (1986) found that pol CMS was temperature stable at low temperatures (22/16^0 C) but temperature sensitive at high temperatures (30/24^0 C). They proposed that temperature affects flower buds before archesporial differentiation occurs. Burns et al. (1991) also while studying the effect of high temperature treatment on maintenance of pol CMS male sterility found that after seven days exposure to 30/24^0 C day/night temperature led to increased pollen production in the F1, with maximum reversion occurring at 6 to 13 days after removal from the high temperature treatment. The results for Mangun mur A. Regent mur A, Karat 7-5 mur A and Karat 7-14 mur A in this study indicate that the male sterility of these lines is not influenced by high

temperature during floral bud development. High temperature sensitivity results in incomplete male sterility that can result in selfing and sibing of the of the sterile lines. Production of hybrids utilizing temperature sensitive CMS A-lines can substantially reduce the hybridity or the proportion of hybrid seed in hybrid seed lots (Pinnisch and McVetty 1990). These authors concluded that reduced hybridity is caused by the production of A-line seed on A-line plants resulting in the presence of male sterile contaminants in the hybrid seed.

Table 6. Mean male sterility index of mur CMS A-lines and a pol CMS A-line grown at 22/16^0 C compared to mur CMS A-lines and a pol CMS A-lines grown at 22/16^{0}C but subjected to 30/24^0 C (day/night) treatment for 7 days at the bud stage

	Growing Conditions	
A-lines	22/16^0 C (Day/night)	30/24^{0}C (Day/night)
	MALE STERILITY INDEX (0 - 6)*	
Mangun mur A	0.0+	0.0 b
Regent mur A	0.0	0.0 b
Karat 7-5 mur A	0.0	0.0 b
Karat 7-14 mur A	0.0	0.0 b
Karat pol A	0.0	2.7 a

Means within a column followed by the same letter are not significantly different as determined by Duncan's multiple Range test at 0.05 probability level.
+ means of 18 plants for all A-lines at each temperature
* 0 = no pollen on any anther, 6 = pollen on all anthers

5.5 CONCLUSIONS

The transfer of mur cytoplasmic male sterility inducing cytoplasm and maintainer genes from Mangun winter rapeseed to summer rape genotypes led to the development of three summer rape mur CMS A-line and mur CMS B-line pairs. The newly developed summer rape lines exhibited summer habit with phenology and agronomic performance similar to their summer rape genetic sources. Male sterility of the mur CMS A-lines was temperature stable up to $30/24^0$ C. The mur CMS A-lines and B-lines appear to have good potential for use in hybrid summer rape cultivar development programmes.

6. COMPARISON OF THE EFFECT OF mur AND nap CYTOPLASMS ON THE PERFORMANCE OF INTERCULTIVAR SUMMER RAPE HYBRIDS

6.1 ABSTRACT

The comparative performance of twelve male fertility restored, hand crossed, summer rape (Brassica napus L.) hybrids in the male sterility inducing mur cytoplasm (from Diplotaxis muralis) (six hybrids), and the nap cytoplasm (six hybrids) was investigated in four Manitoba environments in 1994: (Winnipeg early seeded, Winnipeg late seeded, Portage La Prairie and Carman), to determine the relative effect of these two cytoplasms on hybrid performance. Hybrids in both mur and nap cytoplasms exhibited significant positive high-parent heterosis for seed yield (twelve hybrids), total dry matter (TDM) (nine hybrids) and harvest index (three hybrids) and negative high-parent heterosis for days to flowering (eight hybrids), days to maturity (six hybrids), oil content (five hybrids) and protein content (five hybrids). When combined over cytoplasms and considered in each of four environments, hybrids in mur and nap cytoplasms were not significantly different for days to emergence, seedling vigour, days to flowering and maturity, plant height, seed yield, TDM, or harvest index. The mur and nap cytoplasm hybrids were significantly different in all environments for oil content, and significantly different for protein content in one of four environments. When combined over cytoplasms and environments, hybrids in the mur cytoplasm performed significantly poorer than hybrids in the nap cytoplasm for seed yield, total dry matter, harvest index

and oil content. Hybrids in the mur cytoplasm were, however, significantly better than hybrids in the nap cytoplasm for protein content. Although the results suggest some pleiotropic negative effects (i.e. biological cost) associated with the mur cytoplasm, nonetheless the mur CMS has significant potential for use in commercial hybrid seed production for summer rape hybrid cultivars.

6.2 INTRODUCTION

The development of cultivars with superior characteristics is important to plant breeders, producers and consumers. Hand crossed hybrids in winter and summer forms of oilseed rape have exhibited significant high-parent heterosis (20% to 70%) for seed yield (Hutcheson et al. 1981, 1983, Lefort-Buson and Dattee 1982, Sernyk and Stefansson 1983, Grant and Beversdorf 1985, Brandle and McVetty 1989, McVetty et al. 1990, , Schuler et al. 1992, Falk et al. 1994). Significant high parent heterosis has also been shown for other agronomic characters e.g. plant height, leaf area, disease and lodging resistance (Sernyk and Stefansson 1983) and days to maturity, oil content and oil yield (Schuler et al. 1992, Falk et al. 1994). These observations have created interest in development and utilization of hybrids in summer rape.

The production and utilization of hybrids on a commercial scale requires an effective pollination control system to facilitate commercial hybrid seed production. Several pollination control systems including self-incompatibility (SI), genetic male sterility (GMS), cytoplasmic male sterility (CMS), male gametocides and a "pollen killer" system (now called nuclear male sterility - NMS), have been developed or are under

development (McVetty 1995). Of these, CMS is the most studied and widely used system of pollination control.

Several male sterilizing cytoplasms, which could potentially be developed into fully functional CMS systems, including ogu (Ogura 1968 and Bannerot et al. 1977), nap (Shiga et al. 1983, Thompson 1972), pol (Fu 1981) and mur (Hinata and Konno 1979) have been reported in the Brassica genus.

The ogu male sterile cytoplasm discovered by Ogura (1968) was developed by transferring the genome of Brassica napus into a male sterility inducing cytoplasm of radish (Raphanus sativus L.)(Bannerot et al. 1977). The utilization of ogu was initially limited by the chlorotic nature of male sterile plants at temperatures below 12^0 C, the impairment of female fertility and a lack of restorer genotypes. These deficiencies were corrected through protoplast fusion and transfer of restorer genes from Raphanus sativus (Pelletier et al. 1983, Pelletier 1990 and Delourme et al. 1991 and 1994), leading to development of restorer lines with one restorer gene and normal female fertility. To make the ogu CMS system fully workable, however, double low (low erucic acid, low glucosinolate) restorer lines with normal meiotic behaviour will have to be developed.

The nap male sterile cytoplasm was discovered in F2 generations from intercultivar crosses of several winter and spring cultivars with the cultivar Bronowski as the pollen parent (Shiga et al. 1983, Thompson 1972). The use of the nap cytoplasm in hybrid production is limited by high temperature sensitivity of the A-lines, resulting in reversion to male fertility that could result in selfing and sibling, affecting the hybridity

of hybrid seed lot, and by the very limited number of maintainer genotypes available (Fan et al. 1986).

The pol male sterile cytoplasm was discovered in the B. napus cultivar Polima (Fu 1981). The pol CMS system has also some limitations. Fan et al. (1986) and Burns et al. (1991) reported that pol CMS A-lines reverted to male fertility at 30^0 C. Fu et al. (1990) reported that the pol cytoplasm had no negative effects on the performance of rape hybrids. In contrast, in a comparative study of male fertility restored summer rape hybrids in both pol and nap male sterility inducing cytoplasms, the hybrids in pol cytoplasm were found to yield, on average, 23% less than the identical hybrids in nap cytoplasm and have 3% lower relative oil content (McVetty et al. 1990). Evaluation of the effects of pol and nap cytoplasms on the performance of 3 summer rape derived isoline pairs showed that one pol line yielded significantly less seed (17%) than the corresponding nap line, three pol lines had significantly lower protein content (2 to 3% more relative to their corresponding nap lines) and two pol lines produced significantly less seed energy (4 to 18% less relative to their corresponding nap lines) (McVetty and Pinnisch 1994). These latter studies reported pleiotropic negative effects (i.e. biological costs) associated with the pol cytoplasm. The limitations reported for the currently available CMS systems in rape suggests that a search for, and development of, additional CMS systems is necessary. One of the potential new CMS systems that could be developed is the mur CMS system.

The Diplotaxis muralis male sterile cytoplasm and the derived CMS system (mur) in B. rapa was developed from sand rocket [D. muralis (L) DC], a short lived weed which occurs in annual, biennial or perennial forms and is mainly distributed in Southern and Central Europe (Tutin et al. 1964). Hinata and Konno (1979) reported that a mur CMS line in the B. rapa cultivar Yukina was established by transfer of the B. rapa genome into the cytoplasm of D. muralis by means of repeated back crosses. The male sterile plants were characterized by two nectaries, narrow petals and non dehiscent anthers which contained a small amount of pollen. Shiga (1980) reported that the hybrids between D. muralis and the B. napus cultivar Norin 16 were male sterile and he designated the male sterility system as mur CMS. But Pellan-Delourme and Renard (1987) found that the crosses between cytoplasmic male sterile B. rapa with D. muralis cytoplasm and Norin 16 produced only male fertile plants, indicating that Norin 16 carried restorer genes for D. muralis cytoplasm. The mur cytoplasm was also used in an attempt to produce male sterile plants in B. napus by means of repeated back crosses of the summer rape cultivar Regent into D. muralis containing the mur cytoplasm. The frequency of male sterile plants was low and declined with each successive backcross (Fan et al. 1986). Cytological observation of the male sterile plants indicated that male sterility was associated with an extra chromosome from D. muralis, instead of from a male sterility inducing nuclear-cytoplasm interaction (Fan et al. 1986).

Restorer genes for the mur cytoplasm were found in B. rapa (Shiga 1980). All Canadian and European B. rapa cultivars tested restored male sterility induced by D.

muralis cytoplasm (Fan et al. 1986). Maintainer genotypes are rare in turnip rape. Maintainer genotypes are also non-existent or very low in frequency in summer rape (Pellan-Delourme and Renard 1987). A winter habit oilseed rape cultivar carrying mur cytoplasm and maintainer genes was acquired by the University of Manitoba. Using this source, mur CMS A-lines and their respective B-lines were developed in summer rape. Evaluation of hybrids based on the mur CMS system has not been conducted. The purpose of this study was to determine the relative the effect of mur and nap cytoplasms on the performance of intercultivar summer rape hybrids.

6.3 MATERIALS AND METHODS

Muralis CMS male sterile (mur cytoplasm) A-lines and maintainer (nap cytoplasm) B-lines in summer rape were developed by crossing Mangun mur B-lines with the cultivars Regent and Karat, selfing the F1 to generate the F2, and then test crossing at least 100 F2 plants from each cross to the Mangun mur A-line (See chapter 5). The test cross progeny were grown to identify, and then select, maintainer F2 genotypes. These F2 genotypes were selfed to generate F3 families. Muralis CMS maintainer F3 families with phenotypic and phenological characteristics similar to their summer rape parent, were selected and used to develop mur A-lines and B-lines (in the nap cytoplasm) by back crossing the selected F3 maintainer families to appropriate back cross generation mur A-lines six times. This placed the summer rape nuclear genotypes into the mur cytoplasm

to create mur CMS A-lines and produced isogenic mur CMS maintainer nuclear genotypes (B-lines) in the nap cytoplasm.

Summer rape hybrids were produced in the greenhouse during the winter of 1993 by crossing 3 mur A-lines (Mangun mur A, Regent mur A and Karat mur A) and their respective B-lines, (Mangun mur B, Regent mur B and Karat mur B, all in the nap cytoplasm) with the open pollinated cultivars Regent, Karat and Marnoo. These open pollinated cultivars were selected because intercultivar hybrids derived from them exhibited high parent-heterosis (Sernyk and Stefansson 1983), and because they belong to different heterosis groups (Brandle and McVetty 1989). Regent is from Canada, Karat is from Europe and Marnoo is from Australia. The Mangun mur A-line and Mangun B-line were grown in the growth room for 21 days in flats filled with metro-mix growth media and then vernalized for 30 days at 4° C. After vernalization the seedlings were transplanted into 13 cm fibre pots filled with soil, sand and peat in the ratio 2:1:1, and placed in the greenhouse. The Regent and Karat derived mur A-lines and B-lines and open pollinated cultivars were also grown in the growth room and after 21 days transplanted into 13 cm pots filled with soil, sand and peat in the ratio 2:1:1, and placed in the greenhouse. At flowering, the male sterile mur A-lines and B-lines were crossed as females to the open pollinated cultivars. Twelve hybrids, in three groups of four hybrids each, were produced ([Mangun mur A-line and B-line] x Karat and x Regent), ([Regent mur A-line and B-line] x Karat and x Marnoo) and ([Karat mur A-line and B-line] x Regent and x Marnoo). For the cytoplasmic male sterile mur A-lines, crossing

was accomplished by transfer of pollen from the flowers of the open pollinated male parents to the A-lines, while for the B-lines, the crossing was accomplished by emasculation and subsequent pollination. At least 72 plants of each female parent were used to produce each hybrid.

The four field trials of the above materials consisted of 16 entries; 12 hybrids and 4 open pollinated cultivars. The four trials were grown in 3 locations in Manitoba in 1994: (1) Winnipeg early-seeded, (2) Winnipeg late-seeded, (3) Portage la Prairie and (4) Carman. Seeding was done on May 9 and May 30 for the Winnipeg early seeded and late seeded trials, respectively, on May 26 for the Portage la Prairie trial and on May 25 for the Carman trial.

A randomized complete block design with 4 replications was used for all trials. Entries (i.e. hybrids and open pollinated parents) were considered fixed effects and blocks and environments random. Individual plots were 3 m x 4 rows with a 30 cm spacing between the rows. Seeding was done with a Hege belt cone seeder that placed the seeds at a depth of 2 cm. A seeding rate of 6 kg/ha was used. At seeding, 17-20-0 (N P K) fertilizer with 15% elemental sulphur was applied at a rate of 150 kg N/ha. Granular carbofuran (2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methyl carbamate) was applied with the seed at a rate of 1.0 kg a.i./ha to control flea beetles, Phyllotreta cruciferae (Goeze). To minimize border effects, each trial was bordered on both sides with 4 rows of Regent. The trials were kept free of weeds throughout the growing season by hand weeding.

Growth characteristics including days to emergence, seedling vigour, days to flower, days to physiological maturity (50% of the seed in a plot turn brown or black), lodging and height were recorded for all entries in all plots. The seedling vigour and lodging were visually rated where for seedling vigour, 1 represented least vigourous and 5 most vigourous whereas for lodging 1 represented resistance and 5 susceptibility. At maturity, the entire above ground material of each plot was harvested by cutting at ground level, placing it in burlap sacks and air drying it for several weeks before threshing.

Dried plot materials were weighed to determine the total dry matter (TDM) and threshed using a stationery thresher from which clean seed was obtained and weighed to determine seed yield. Final seed yields and TDM yields (kg/ha) were determined from the respective weights. Harvest index was determined by dividing seed yield by TDM. Seed samples from each plot were analyzed for oil and protein content. Oil content was determined by Nuclear Magnetic Resonance following the Federation of Oils and Fats Association (FOSTA) method for rapeseed using 25 g of seed at 0% moisture (Robertson and Morrison 1979). Protein content was determined using the total combustion method (AOCS Official Method Ba 4e-93), with results presented as %N x 6.25.

Analysis of variance was performed using the SAS statistical package. The trials were combined over all environments or cytoplasms (mur or nap) because the entry x environment, environment x cytoplasm or entry x cytoplasm interaction for the combined trails was found to be non significant or very small for all traits. Mean comparison tests

were determined using student's t test at the 0.05 probability level of significance or Duncan's Multiple Range Test at the 0.05 probability level of significance.

Hybrids and open pollinated parents were compared for trials combined over environments. In addition, hybrids combined over cytoplasms were compared at each environment and combined over environments.

High-parent heterosis was calculated by comparing the performance of the hybrid and its respective high performing parent in the cross for all traits. Relative difference in performance in percent between the cytoplasms was estimated by:(value of mur - value of nap)/ value of nap x 100 while the relative difference to determine the advantage or disadvantage of mur or nap cytoplasm was estimated by:(value of nap - value of mur)/value of nap x 100. The high-parent heterosis, the relative difference of the cytoplasms and the relative advantage or disadvantage of the cytoplasm were considered significant if the analysis of variance showed that the means were significantly different.

6.4 RESULTS AND DISCUSSION

The trials were combined over all environments because the entry x environment interaction for the combined trials was found to be non significant or very small for all traits. Growth characteristics, agronomic and quality data for the trials combined over environments are presented in Tables 1 and 2. High-parent heterosis results for all traits for the trials combined over environments is presented in Tables 3 and 4.

6.4.1 Days to emergence

Days to emergence ranged from a low of 8.1 to a high of 9.7 days (Table 1). There were no significant differences in days to emergence between mur hybrids and nap hybrids for five of six hybrid pairs. The Regent x Karat hybrids were, however, significantly different in days to emergence, with the mur hybrid being 0.5 days earlier than the nap hybrid. There is therefore, no evidence from these trials, of any negative effects (i.e. biological costs) associated with the mur cytoplasm for days to emergence in summer rape hybrids.

Two hybrids, one in the mur cytoplasm and one in the nap cytoplasm, showed significant positive heterosis for earliness (i.e. they were earlier in days to emergence than their earlier parent), while two hybrids, both in the mur cytoplasm, showed significant negative heterosis for earliness, (i.e. they were later in days to emergence than their earlier parent (Table 3). The majority of the hybrids in either the mur or nap cytoplasms displayed no high parent heterosis for days to emergence, indicating that there would be little improvement in days to emergence to be gained by growing hybrids.

6.4.2 Seedling vigour

Seedling vigour ranged from a low of 3.1 to a high of 4.4 (Table 1). The mur and nap hybrids were not significantly different for seedling vigour for all six hybrid pairs, indicating that the mur and nap cytoplasms provided equal opportunities for expression of seedling vigour in these trials. None of the hybrids in either cytoplasm displayed

significant high-parent heterosis for seedling vigour (Table 3). This was unexpected since hybrids frequently display significant levels of seedling vigour (Allard 1960).

6.4.3 Days to flower

Days to flower ranged from a low of 44.5 days to a high of 63.6 days (Table 1). The semi-winter habit cultivar Mangun was the latest to flower. The mur and nap hybrids were significantly different in days to flower for three of the six hybrid pairs. For these later three hybrid pairs, the mur cytoplasm hybrids were consistently greater in days to flower than the nap hybrids, by approximately 0.8 days. The mur cytoplasm may, therefore have some delaying effect in the time required to reach flowering, but even in cases where this occurs, the delay is less than one day, of minimal practical significance.

Eight of twelve hybrids displayed significant negative high-parent heterosis [= early], (i.e. they were greater in days to flower than their respective early parents) (Table 3). Even though this was the case, all twelve hybrids were intermediate in days to flower between the two parents used in the respective crosses. These findings are in agreement with Sernyk and Stefansson (1983).

6.4.4 Days to maturity

Days to maturity ranged from a low of 89.9 days to a high of 98.8 days (Table 1). The mur and nap hybrids were not significantly different in days to maturity for five of six hybrid pairs. In the Karat x Marnoo hybrids, the nap hybrid was significantly earlier to maturity, (by 1.9 days) than the mur hybrid. In the majority of cases then, the

mur cytoplasm did not have any negative effects (i.e. biological costs) on days to maturity.

Significant negative high-parent heterosis [=early] was observed for six out of the twelve hybrids (i.e. they were significantly greater in days to maturity than their early parent) (Table 3). Six hybrids displayed no significant high-parent heterosis for days to maturity. These hybrids were intermediate in maturity between the two parents used in the respective crosses, or approximately 1 day later to maturity than their later parent.

6.4.5 Plant height

Plant height ranged from 111.6 cm to 137.4 cm (Table 1). The mur hybrids and nap hybrids were not significantly different in height for three hybrid pairs while the mur and nap hybrids were significantly different in height for three hybrid pairs. The mur cytoplasm hybrids were taller in two of these latter three hybrid pairs. There is, therefore no evidence of negative effects (i.e. biological costs) of the mur cytoplasm on plant height. Seven of twelve hybrids displayed significant high-parent heterosis [=tall] for plant height (Table 3). This was expected since hybrids are frequently taller than their taller parent (Allard 1960).

6.4.6 Lodging

The range in lodging in these trials was small, varying from 1.6 to 1.9 (Table 1). The mur and nap hybrids were, nonetheless, significantly different in lodging resistance in three hybrid pairs, and in all three cases the nap hybrids displayed less lodging than

their respective mur hybrids. There may be, therefore be some minor negative effects (i.e. biological costs) on lodging related to the mur cytoplasm.

Significant high-parent heterosis [=less lodging] for lodging was observed for the three nap hybrids noted above (Table 3). No significant high parent heterosis for lodging was displayed by the other nine hybrids, indicating that there would be little improvement in lodging resistance to be gained by growing hybrids.

6.4.7 Seed Yield

Seed yield ranged from a low of 644 kg/ha to a high of 2408 kg/ha (Table 2). All twelve hybrids displayed superior seed yields compared to their better parents. The mur and nap hybrids were significantly different for seed yield in two of six hybrid pairs, and in these two hybrid pairs, the mur hybrid yielded significantly less than the corresponding nap hybrid. There is, therefore a negative effect of the mur cytoplasm (i.e. biological cost) on seed yield, at least in some hybrid genotypes.

All twelve hybrids displayed significant high parent heterosis for seed yield (Table 4). Sernyk and Stefansson (1983) using intercultivar summer rape hybrids found high parent heterosis of 38% and 43% for seed yield from hybrids between Marnoo and Regent and Karat and Regent. Brandle and McVetty (1989) found that inbred-line-derived summer rape hybrids exhibited high parent heterosis for seed yield of up to 120%.

The level of high-parent heterosis for seed yield was less for the mur hybrids in five of six hybrid pairs. There is, therefore, a biological cost associated with the mur cytoplasm. Even with this biological cost, however, mur cytoplasm hybrids still exhibited

significant high-parent heterosis for seed yield that can be exploited in production of commercial summer rape hybrids. This parallels the findings of McVetty et al. (1990) who found that the pol cytoplasm also had a biological cost for seed yield, but that the levels of high parent-heterosis for seed yield observed in pol hybrids were still sufficient to permit commercial use.

6.4.8 Total dry matter

Total dry matter ranged from a low of 6693 kg/ha to a high of 11490 kg/ha (Table 2). Nine hybrids produced significantly more TDM than their better parents. There were no significant differences in TDM for five of six hybrid pairs (Table 1). The Mangun X Karat hybrids displayed significant differences in TDM. In this hybrid pair, the mur hybrid produced significantly less TDM than the nap hybrid. There may be some negative effects (i.e. biological costs) of the mur cytoplasm on TDM in summer rape hybrids, at least for some genotypes.

Nine of twelve hybrids displayed significant high parent heterosis for TDM (Table 4). Sernyk and Stefansson (1983) observed high parent-heterosis for TDM in summer rape hybrids. In one hybrid pair, the mur hybrid did not display high parent heterosis for TDM while the nap hybrid did display significant high parent heterosis for TDM, an indication that the mur cytoplasm may have some negative effects (i.e. biological costs) on TDM production in summer rape hybrids. This parallels the findings of McVetty et al. (1990) who found that the pol cytoplasm also had a biological cost for TDM production in summer rape hybrids.

6.4.9 Harvest index

Harvest index ranged from a low of 9.1% to a high of 22.7% (Table 2). The mur and nap hybrids were not significantly different in harvest index for five of six hybrid pairs. The mur hybrid was significantly lower in harvest index than the nap hybrid in the Mangun x Regent hybrids. There may be some negative effects (i.e. biological costs) of the mur cytoplasm on harvest index in summer rape hybrids, at least for some genotypes.

Significant high-parent heterosis for harvest index was observed in three hybrids, all in the nap cytoplasm (Table 4). Sernyk and Stefansson (1983) observed high-parent heterosis for harvest index in summer rape hybrids. The mur hybrids displayed non significant high-parent heterosis for harvest index in the three hybrid pairs where the nap hybrids displayed significant high-parent heterosis for harvest index, again indicating that the mur cytoplasm has some negative effects, i.e. biological costs for harvest index in summer rape hybrids. These results are in agreement with McVetty et al. 1990) who found that the pol cytoplasm had negative effects on harvest index in summer rape hybrids.

6.4.10 Oil content

Oil content ranged from 42.4% to 47.4% (Table 2). Hybrids in mur cytoplasm exhibited significantly lower oil content than their respective hybrids in nap cytoplasm for five of six hybrid pairs. In the sixth hybrid pair, the mur hybrid was again lower in oil content than the nap hybrid, but not significantly so. There is clearly a substantial negative effect (i.e. biological cost) of the mur cytoplasm on oil content in the hybrids.

This parallels the findings of McVetty et al. (1990) who found that the pol cytoplasm had substantial negative effects on oil content in pol cytoplasm summer rape hybrids.

Significant negative high-parent heterosis was observed in five out of the twelve hybrids (Table 4). Falk (1994) found negative high parent-heterosis of as much as 4.7% for oil content in summer turnip rape hybrids. Brandle and McVetty (1989) reported significant negative high-parent heterosis for oil content in summer rape hybrids. Grant and Beversdorf (1985) reported little or no high-parent heterosis for oil content in summer rape hybrids. Of the five hybrids which displayed significant negative high parent-heterosis for oil content in this study, all five hybrids were in the mur cytoplasm. There are, therefore large biological costs on oil content related to the mur cytoplasm evident in this study. This creates a disadvantage for mur hybrids compared to nap hybrids that may limit the usefulness of the mur CMS system in summer rape hybrid production. The extent of this problem, and the extent to which this problem can be ameliorated, needs to be determined before the mur CMS system can be recommended for use in hybrid summer rape cultivar development programs.

6.4.11 Protein content

Protein content ranged from 23.6% to 25.5% (Table 2). The mur hybrids were not significantly different for protein content from the nap hybrids in five of six hybrid pairs. In the Karat x Marnoo hybrids, the mur hybrid was significantly higher in protein content than the nap hybrid. There is, therefore little effect of the mur cytoplasm on protein

content in summer rape hybrids. This agrees with McVetty et al. (1990) who found the same result for pol cytoplasm summer rape hybrids.

Significant negative high-parent heterosis for protein content was observed for five hybrids while seven hybrids displayed no significant high-parent heterosis for protein content (Table 4). Therefore, hybrids can not be used to increase protein content in summer rape.

6.4.12 Effect of environment

The hybrids were combined over cytoplasm within trials to study the effect of environment on mur and nap hybrid group performance. The results of these analyses are shown in Tables 5 and 6. The mur hybrid group was not significantly different from the nap hybrid group for days to emergence, seedling vigour, days to flower, days to maturity, plant height or lodging in any environment (Table 5). Similarly, the mur hybrid group was not significantly different from the nap hybrid group for seed yield, TDM or harvest index in any of the environments (Table 6), or for protein content in three out of four environments (Table 6). In contrast, the mur hybrid group was significantly lower in oil content than the nap hybrid group in all four environments. The relative effects of the mur and nap cytoplasms on hybrid performance were, therefore consistent over environments and the differences in performance between hybrids in the two cytoplasm was non significant for all traits except protein content and oil content. Protein content was significantly higher for the mur hybrid group compared to the nap hybrid group in only one of four environments. There was, therefore a cytoplasm x environment

interaction observed for protein content in this study. These results for oil and protein contents agree with McVetty et al. (1990) who found that for summer rape hybrids, the pol cytoplasm had negative effects (i.e. biological costs) on oil content and occasional positive effects on protein content, compared to the nap cytoplasm.

6.4.13 Effect of cytoplasm

To further investigate the effect of the mur and nap cytoplasms on hybrid performance, hybrids were combined over cytoplasms and the trials were combined over environments. The results of these analyses are presented in Tables 7 and 8. The average high parent heterosis for the mur and nap hybrid groups combined over environments was also calculated. The results of these analyses are presented in Table 9.

Averaged over cytoplasms and environments, there were no significant differences between the mur hybrid group and the nap hybrid group for days to emergence, seedling vigour, days to flower, days to maturity, plant height or lodging (Table 7).

In contrast, the mur hybrid group had significantly lower seed yield, lower TDM, lower harvest index and lower oil content than the nap hybrid group (Table 8). The situation is reversed for protein content, however, where the mur hybrid group had significantly higher protein content than the nap hybrid group. Similar observations were made by McVetty et al. (1990) on the effect of pol cytoplasm compared to the nap cytoplasm on seed yield, TDM, harvest index, oil content and protein content in summer rape hybrids.

The mur cytoplasm hybrid group yielded 8% less than the nap hybrid group, produced 5% less TDM than the nap hybrid group, had a harvest index 4% lower than the nap hybrid group, and had an oil content 2.9% lower than that for the nap hybrid group (Table 8). In contrast, protein content of the mur hybrid group was 3% higher than for the nap hybrid group (Table 8). These results parallel those of McVetty et al. (1990), where it was reported that hybrids in the pol cytoplasm were found to produce on average 23% less seed yield than the identical hybrids in nap cytoplasm, and have a 3% lower relative oil content. McVetty and Pinnisch (1994) also evaluated the effects of pol and nap cytoplasms on the performance of 3 oilseed-rape-derived isoline pairs found that one pol line yielded significantly less seed (17%) than the corresponding nap line, three pol lines had significantly higher protein content (2 to 3% more relatively than their corresponding nap lines and two pol lines produced significantly less seed energy (4 to 18% more relatively than their corresponding nap lines. These studies reported pleiotropic negative effects (i.e. biological costs) associated with the pol cytoplasm. In this study, there is evidence of negative effects, i.e. biological costs associated with the use of the mur cytoplasm in summer rape hybrids.

The mur hybrid group and the nap hybrid group were significantly different in high parent-heterosis for seed yield, TDM, harvest index, oil content and protein content (Table 9). The mur hybrid group displayed 37% less high-parent heterosis for seed yield, 26% less high-parent heterosis for TDM, and 54% less high-parent heterosis for harvest index, compared to the nap hybrid group (Table 9). For the two traits where significant

negative high-parent heterosis was observed, the mur hybrid group displayed 357% more negative high-parent heterosis, relatively, for oil content than the nap hybrids group, while the mur hybrid group displayed 62% less negative high-parent heterosis, relatively, for protein content than the nap hybrid group. The reduced high-parent heterosis observed for seed yield and the increased negative high-parent heterosis for oil content for the mur hybrid group compared to the nap hybrid group is a further reflection of the negative effects, i.e. biological costs of the mur cytoplasm and CMS system.

6.5 CONCLUSIONS

The development and utilization of hybrids in summer rape requires an effective pollination control system, for example a CMS system comprised of stable male sterile A-lines, corresponding B-lines and R-lines, to facilitate the production of male fertile hybrids. In this study, summer rape hybrids produced in both the mur and nap cytoplasms exhibited significant seed yield and protein content advantages over their better parents. The mur hybrids as a group are, however, significantly lower yielding than the nap hybrids as a group. The mur hybrids as a group also produce significantly less TDM, have a significantly lower harvest index and have significantly lower oil content than the nap hybrid as a group. However the mur hybrid group produced significantly higher protein content than the nap hybrid group, offsetting, at least in part, the oil content disadvantage displayed by the mur hybrid group. Although it appears that mur cytoplasm and the derived CMS system can be used to produce summer rape hybrids

which have significant high parent-heterosis for seed yield, the biological costs associated with the mur cytoplasm compared to the nap cytoplasm will make the breeding of successful mur CMS hybrids more difficult. It would still be more desirable to produce summer rape hybrids using the nap cytoplasm rather than the mur system. However, the problems associated with nap CMS system have to be overcome before its utilization becomes a reality. In the meantime, the mur cytoplasm and the derived mur CMS system has significant potential for use in the production of hybrid summer rape cultivars.

Table 1. Days to emergence seedling vigour, days to flowering and maturity, height and lodging for summer rape hybrids and parents for trials combined over four environments

ENTRIES	EMERGENCE (Days)	VIGOUR (1-5)	FLOWERING (Days)	MATURITY (Days)	HEIGHT (cm)	LODGING (1-5)
Mangun mur A x Karat	8.4 cdef[1]	4.1 abc	49.9 b	95.5 b	131.3 b	1.9 a
Mangun nap x Karat	8.5 cde	4.0 abc	49.1 c	95.3 b	137.3 a	1.6 b
Mangun mur A x Regent	8.4 cde	4.2 abc	47.7 d	94.7 bc	130.1 bc	1.8 a
Mangun nap x Regent	8.4 cde	4.4 abc	47.3 d	94.1 bc	127.8 bcd	1.6 b
Regent mur A x Karat	8.6 c	3.9 abc	46.3 ef	92.6 e	127.6bcde	1.9 a
Regent nap x Karat	9.1 b	4.1 abc	46.2 efg	92.8 ed	130.3 b	1.9 a
Regent mur A x Marnoo	8.6 cd	4.2 abc	45.8 fg	92.2 efg	121.6 fgh	1.9 a
Regent nap x Marnoo	8.4 cde	4.1 abc	45.7 g	91.4 fgh	123.6defg	1.8 a
Karat mur A x Regent	8.2 ef	4.3 ab	45.1 h	91.1 h	128.8 bc	1.8 a
Karat nap x Regent	8.3 def	4.3 ab	44.5 i	90.9 hi	122.8efgh	1.6 b
Karat mur A x Marnoo	8.4 def	4.4 a	47.6 d	93.8 cd	127.2bcde	1.9 a
Karat nap x Marnoo	8.1 f	4.3 ab	46.4 e	91.9 efgh	121.7 fgh	1.8 a
Regent (opp)	8.3 cdef	4.4 a	45.1 hi	89.9 i	119.1 gh	1.8 a
Karat (opp)	9.3 b	3.6 cd	47.3 d	92.3 ef	118.0 h	1.8 a
Marnoo (opp)	8.3 cdef	3.8 d	47.4 d	91.2 gh	111.6 i	1.9 a
Mangun (opp)	9.7 a	3.1 e	63.6 a	98.8 a	125.1cdef	1.8 a
GRAND MEAN	8.6	4.1	47.8	93	125.2	1.8
C.V. (%)	6.1	8.4	1.6	1.7	5.73	23.1

[1] Means within a column followed by the same letter are not significantly different as determined by Duncan's Multiple Range Test at 0.05 probability level.
opp = Open pollinated population

Table 2. Seed yield, total dry matter, harvest index, oil content and protein content for summer rape hybrids and parents for trials combined over four environments

ENTRIES	SEED YIELD (kg/ha)	TOTAL DRY MATTER kg/ha)	HARVEST INDEX (%)	OIL (%)	PROTEIN (%)
Mangun mur A x Karat	1887 ef[1]	9870 de	19.6 f	45.8 edf	24.5 bc
Mangun nap x Karat	2273 abc	11490 a	20.0 def	45.6 abcd	24.2 bcde
Mangun mur A x Regent	1998 def	10295 bcd	19.7 ef	46.1 cde	24.1 bcde
Mangun nap x Regent	2317 ab	10943 abc	21.6 abc	47.2 ab	23.8 cde
Regent mur A x Karat	2161 abcd	10009bcde	21.6 abc	45.4 efg	24.8 abc
Regent nap x Karat	2408 a	10977 ab	22.0 ab	46.8 abc	24.1 bcde
Regent mur A x Marnoo	2044 cde	9882 cde	21.0 bcde	44.4 h	24.5 bcd
Regent nap x Marnoo	2140 bcd	9810 de	21.9 b	46.3 bcde	23.6 ed
Karat mur A x Regent	2194 abcd	10260 bcd	21.7 abc	46.2 cde	24.2 bcde
Karat nap x Regent	2308 ab	10290 bcd	22.7 a	47.4 a	23.6 e
Karat mur A x Marnoo	2122 bcde	10130 bcd	21.0 bcde	44.8 hg	24.8 ab
Karat nap x Marnoo	2050 cde	9826 de	20.8 bcde	46.5 bcd	23.5 e
Regent (opp)	1789 fg	8978 ef	20.4 cde	47.1 ab	24.4 bcde
Karat (opp)	1604 g	8438 f	19.1 f	46.6 abcd	24.5 bcd
Marnoo (opp)	1630 g	8172 f	20.2 cdef	44.9 hg	24.8 ab
Mangun (opp)	644 h	6693 g	9.6 g	42.4 i	25.5 a
GRAND MEAN	1973	9754	20.1	45.9	23.8
C.V (%)	18.2	15.7	10.1	2.8	5.8

[1] Means within a column followed by the same letter are not significantly different as determined by Duncan's Multiple Range Test at 0.05 probability level.
opp = Open pollinated population

Table 3. High parent heterosis for days to emergence, flowering and maturity, seedling vigour, height, and lodging for summer rape hybrids for trials combined over four environments

	HIGH PARENT HETEROSIS (%)					
ENTRIES	EMERGENCE	VIGOUR	FLOWERING	MATURITY	HEIGHT	LODGING
Mangun mur A x Karat	9.7 *[1]	13.9	-5.5 *	-3.6 *	4.8 *	-5.6
Mangun nap x Karat	8.7 *	11.1	-3.8 *	-3.0 *	9.6 *	11.1 *
Mangun mur A x Regent	-1.2	-4.6	-5.8 *	-5.3 *	4.0	0.0
Mangun nap x Regent	-1.2	0.0	-4.9 *	-4.7 *	2.4	11.1 *
Regent mur A x Karat	-3.6	-9.1	-2.7 *	-3.0 *	7.6 *	-5.6
Regent nap x Karat	-9.6 *	-6.8	-2.4 *	-3.2 *	9.2 *	-5.6
Regent mur A x Marnoo	-3.6 *	-4.6	-1.6 *	-2.6	2.5	-5.6
Regent nap x Marnoo	1.2	-6.8	-1.3 *	-1.7	4.2 *	0.0
Karat mur A x Regent	-1.2	-2.2	0.0	-1.3	8.4 *	0.0
Karat nap x Regent	0.0	-2.2	1.3	-1.1	3.4	11.1 *
Karat mur A x Marnoo	1.2	15.8	-0.6	-2.8	7.6 *	-5.6
Karat nap x Marnoo	2.4	13.2	2.1	-0.8	3.4	0

[1] = Significant at 0.05 probability level
TDM = Total dry matter
HI = Harvest index

Table 4. High parent heterosis for seed yield, total dry matter, harvest index, percent oil content and protein content for summer rape hybrids for trials combined over four environments

	HIGH PARENT HETEROSIS (%)				
ENTRIES	SEED YIELD	TDM	HI	OIL	PROTEIN
Mangun mur A x Karat	17.6 *[1]	17.0 *	2.6	-1.7	-3.9 *
Mangun nap x Karat	42.7 *	36.2 *	4.7	-2.2	-5.1 *
Mangun mur A x Regent	11.7 *	14.7 *	-3.4	-2.1 *	-1.6 *
Mangun nap x Regent	29.5 *	21.9 *	5.9	0.02	-6.7 *
Regent mur A x Karat	20.8 *	11.5	5.9	-3.6 *	-2.9
Regent nap x Karat	34.6 *	22.3 *	7.8 *	-0.6	-1.6
Regent mur A x Marnoo	14.3 *	10.1	2.9	-5.7 *	-1.2
Regent nap x Marnoo	19.6 *	9.3	7.4 *	-1.6	-4.8
Karat mur A x Regent	22.6 *	14.3 *	6.4	-1.9 *	-1.3
Karat nap x Regent	29.0 *	14.6 *	11.3 *	0.6	-3.7
Karat mur A x Marnoo	30.3 *	20.6 *	3.9	-3.9 *	0.0
Karat nap x Marnoo	26.0 *	16.5 *	3.0	-0.2	-5.2*

*[1] = Significant at 0.05 probability level

Table 5. Effect of environment on days to emergence, flowering and maturity and seedling vigour, height and lodging for mur and nap summer rape hybrid groups grown in four environments

	EMERG (Days)		VIG (1-5)		FLOW (Days)		MAT (Days)		HGT (cm)		LOG (1-5)	
ENVIRONMENT	mur	nap	mur	nap	mur	nap	mur	nap	mur	nap	mur	nap
WINNIPEG 1	7.7 a[1]	7.8 a	3.8 a	3.9 a	47.8 a	47.5 a	93.3 a	93.0 a	110 a	109 a	1.2 a	1.1 a
WINNIPEG 2	7.0 a	7.1 a	3.9 a	4.0 a	52.2 a	51.0 a	97.3 a	96.1 a	116 a	117 a	1.3 a	1.1 a
PORTAGE	7.0 a	7.0 a	5.0 a	5.0 a	45.0 a	44.7a	94.6 a	94.5 a	153 a	152 a	3.9 a	3.6 a
CARMAN	12.0 a	12.0 a	4.0 a	4.0 a	43.2 a	42.8 a	88.0 a	87.2 a	131 a	131 a	1.0 a	1.1 a

[1] Means in a row within a trait are not significant different as determined by t test at 0.05 probability level

1 = Early Seeded
2 = Late Seeded

Emerg = Emergence
Vig = Vigour
Flow = Flowering
Mat = Maturity
Log = Lodging

Table 6. Effect of environment on seed yield, total dry matter, harvest index, oil content and protein content for mur and nap summer rape hybrid groups grown in four environments

ENVIRONMENT	SEED YIELD (kg/ha)		TDM (kg/ha)		HARVEST INDEX (%)		OIL (%)		PROTEIN (%)	
	mur	nap	mur	nap	mur	nap	mur	nap	mur	nap
WINNIPEG 1	2152 a[1]	2309 a	10256 a	10402 a	21.2 a	21.2 a	47.3 b	48.3 a	22.1 a	21.5 a
WINNIPEG 2	1774 a	1860 a	8492 a	9134 a	20.9 a	20.6 a	46.0 b	47.4 a	23.4 a	22.8 a
PORTAGE	2405 a	2640 a	12593 a	13081 a	19.2 a	20.2 a	43.9 b	45.2 a	25 3 a	24.9 a
CARMAN	1940 a	2190 a	9057 a	9607 a	21.4 a	22.9 a	44.7 b	46.3 a	25.2 a	24.0 b

[1] Means in a row within a trait are not significant different as determined by t test 0.05 probability

1 = Early Seeded
2 = Late Seeded

TDM = Total Dry Matter

Table 7. Comparison of mur and nap summer rape hybrid groups for days to emergence, flowering and maturity, seedling vigour, height, and lodging for trials combined over four environments

ENTRIES	EMERG (Days)	VIG (1-5)	FLOW (Days)	MAT (Days)	HGT (cm)	LOG (1-5)
mur hybrid group	8.4 a[1]	4.1 a	47.0 a	93.3 a	128 a	1.9 a
nap hybrid group	8.5 a	4.2 a	46.5 a	92.7 a	127 a	1.7 a
R.D. (%)	-0.5	-2.4	-1.1	-0.6	0.4	8.2
C.V. (%)	7.3	7.9	4.1	2.4	6	24.9

[1] Means in column followed by the same letter are not significantly different at 0.05 probability level
R.D. = Relative difference
Emerg = Emergence
Vig = Vigour
Flow = Flowering
Mat = Maturity
Hgt = Height
Log = Lodging

Table 8. Comparison of mur and nap summer rape hybrid groups for seed yield, total dry matter, harvest index, oil content and protein content for trials combined over four environments

ENTRIES	SEED YIELD (kg/ha)	TDM (kg/ha)	HI (%)	OIL (%)	PROTEIN (%)
mur hybrid group	2068 b[1]	10074 b	20.7 b	45.4 b	24.0 a
nap hybrid group	2250 a	10556 a	21.5 a	46.8 a	23.3 b
R.D. (%)	-8 *	-5 *	-4 *	-2.9 *	3 *
C.V. (%)	18.3	15.7	10.6	2.8	5.9

[1] Means in column followed by the same letter are not significantly different at 0.05 probability level
* = Significant at 0.05 probability level
R.D. = Relative difference
TDM = Total Dry Matter
HI = Harvest Index

Table 9. Comparison of mur and nap summer rape hybrids for high parent heterosis for days to emergence, seedling vigour, flowering and maturity, height, lodging, seed yield, total dry matter, harvest index, oil content and protein content for trials combined over four environments

ENTRIES	MEAN HIGH PARENT HETEROSIS (%)										
	EMERG	VIG	FLOW	MAT	HGT	LOG	SEED YIELD	TDM	HI	OIL	PROT
mur hybrid group	0.22	-1.5	-2.7	-3.1	5.8	-3.7	19.6	14.7	3.1	-3.2	-1.8
nap hybrid group	0.25	-1.4	-1.5	-2.4	5.4	-4.6	31.1	20.1	6.7	-0.7	-4.7
R.D. (%)	-12	7	80	29	20	-20	-37 *	-26 *	-54 *	357 *	-62 *

* = Significant at 0.05 probability level
Emerg = Emergence
Vig = Vigour
Flow = Flowering
Mat = Maturity
Hgt = Height
Log = Lodging
TDM = Total Dry Matter
HI = Harvest Index
Prot = Protein
R.D = Relative difference

7. GENERAL DISCUSSION AND CONCLUSIONS

The development and utilization of hybrids in summer rape has been stimulated by reports of economically exploitable high-parent heterosis in the hybrid progeny (Sernyk and Stefansson 1983, Brandle and McVetty 1989). To utilize this heterosis, an effective pollination control system, for example a CMS system, comprised of stable male sterile A-lines, corresponding maintainer B-lines and restorer R-lines, is required to permit commercial hybrid seed production. A number of CMS systems including the nap, ogu and pol systems have been reported in summer rape, but they all have limitations, including instability of male sterility at moderate to high temperatures (Fan et al. 1986 and Burns et al. 1991), lack of good maintainers and/or pleiotropic negative effects (i.e. biological costs) of the CMS system on the performance of the hybrids (Fan et al. 1986, McVetty et al. 1990 and McVetty and Pinnisch 1994), necessitating a search for additional CMS systems in summer rape.

Diplotaxis muralis (L) DC. CMS system (mur) A-lines and their corresponding B-lines were developed in summer rape and then evaluated. The results indicated that the mur cytoplasm had some associated undesirable biological costs. Nevertheless, the mur CMS system appears to have good potential for use in hybrid summer rape cultivar development programmes.

To facilitate the development of mur CMS A-lines and B-lines, an attempt to locate maintainer genotypes in summer rape was undertaken. No maintainer genotypes were found in any of 101 summer lines and cultivars evaluated, i.e. they were all mur

CMS system restorers. Therefore, the frequency of occurrence of mur CMS maintainer genotypes in summer oilseed rape is very low, possibly zero. Pellan-Delourme and Renard (1987) similarly found, that only two cultivars out of 147 winter rape genotypes they evaluated were mur CMS maintainer genotypes. The search for maintainer genotypes, or the development of maintainer genotypes via crosses of the winter habit rapeseed cultivar Mangun, or other mur CMS maintainer sources, into summer rape will be required to produce mur CMS A-lines and B-lines. The ready availability of mur CMS restorer genotypes in summer rape indicates that the development of R-lines will not present any problems.

The inheritance study showed that one to three genes controlled the maintenance and restoration of male sterility for the mur CMS system, and that cultivars differed in the number of genes they carried for maintenance and restoration. Shiga (1980) reported that two dominant genes controlled the restoration of mur CMS in B. rapa. The use of summer rape maintainer genotypes with as many as three genes, as found in this study would complicate the development of mur CMS A-lines and B-lines. However, the ready availability of restorer genotypes should compensate for the difficulties in A-line and B-line development and permit the rapid and easy development of large numbers of male fertility restored mur CMS summer rape hybrids.

The transfer of mur cytoplasmic male sterility inducing cytoplasm and maintainer genes from the winter rapeseed cultivar Mangun to summer rape genotypes led to the development of three summer rape mur CMS A-line and B-line pairs. The newly

developed summer rape A-lines and B-lines exhibited summer habit with phenology and agronomic performance similar to their summer rape sources.

The success in development of mur CMS A-lines and B- lines showed that despite the lack of summer rape maintainer genotypes from which mur CMS A-lines and B-lines could be developed, it was nonetheless possible to develop these A-lines and B-lines via crosses to the winter rapeseed cultivar Mangun, followed by back crosses to the recurrent summer rape cultivars. This approach can be applied to develop more A-lines and B-lines to broaden the genetic base of mur CMS system in summer rape.

One of the problems encountered with the nap and the pol CMS systems is their A-line sensitivity to moderate and high temperature treatment (Fan and Stefansson 1986, Burns et al. 1991,) resulting in reversion of the male sterile lines to male fertility. The reversion to male fertility results in selfing and sibing of the A-line plants, which lowers the hybridity of the hybrid seed lots. The male sterility of the newly developed mur CMS A-lines in summer rape was found to be temperature stable up to $30/24^0$ C. The "deep" (completely sterile) and stable male sterility exhibited by the mur CMS A-lines suggest the mur CMS system has good potential for use in hybrid summer rape cultivar development programmes.

The comparative performance of male fertility restored hybrids in the mur and nap cytoplasms was investigated to determine the relative effects on hybrid performance of the mur and nap cytoplasms, respectively. Hybrids in both mur and nap cytoplasms exhibited superior relative performance for seed yield compared to their open pollinated

population parents. Similar results were reported by Sernyk and Stefansson (1983) for summer rape intercultivar hybrids, and by McVetty et al. (1990) for summer rape hybrids in pol and nap cytoplasms. The superiority for seed yield exhibited by hybrids in mur and nap cytoplasms can be exploited through the production of hybrid summer rape cultivars.

Averaged over environments, hybrids in the mur cytoplasm performed significantly poorer than hybrids in the nap cytoplasm for seed yield, total dry matter and oil content. In contrast, the mur hybrids performed significantly better than the nap hybrids for protein content. These results indicated that there are negative effects (i.e. biological costs) associated with the mur cytoplasm. Although it appears that mur cytoplasm and the derived mur CMS system can be used to produce summer rape hybrids which have significant high-parent heterosis for seed yield, the biological costs associated with the mur cytoplasm, compared to the nap cytoplasm, will make the breeding of successful mur CMS summer rape hybrids difficult. It would still be more desirable to produce summer rape hybrids using the nap CMS system rather than the mur CMS system. However, the problems associated with the nap CMS system will have to be overcome before its utilization as a pollen control mechanism becomes a reality. In the meantime, the mur cytoplasm and the derived mur CMS system has good potential for use in the production of hybrid summer rape cultivars. The development of the mur CMS system in summer rape has created new germplasm and new information. This should stimulate further research into the development of the mur CMS system and lead to the

production of summer rape hybrids that will sustain the competitive position of canola in the world's oilseed industry.

8. RECOMMENDATIONS

The results obtained in this study indicate that maintainer genotypes for the mur male sterility inducing cytoplasm are rare or non-existent in summer rape. It was possible, nonetheless, to develop the mur CMS system in summer rape. The mur system A-lines were found to be temperature stable. Hybrids based on the mur CMS system exhibited high parent heterosis, however, there was some biological cost associated with the mur CMS system. Even though this study showed that the mur CMS system has potential for commercial hybrid summer rape production, more research on, and development of, the mur CMS system is necessary, hence the following recommendations:

1. Further field trials of hybrids in mur and nap cytoplasms should be conducted to confirm the respective cytoplasm effects on hybrid performance.
2. Continue the search for mur CMS B-line genotypes in summer rape to broaden the genetic base of mur CMS maintainers.
3. Further development and evaluation of mur CMS A-lines and B-lines should be done to facilitate hybrid summer rape cultivar development. The development of canola quality A-lines and B-lines should be the first priority.
4. Further development and evaluation of mur CMS restorer lines should be done to facilitate hybrid summer rape cultivar development.

5. Molecular and cytological studies to further characterize the mur CMS A-lines, B-lines, R-lines and hybrids.

6. Initiate a study of the disease susceptibility and insect susceptibility related to the mur cytoplasm and derived CMS system.

7. Initiate a study of the floral characteristics of the mur A-lines, especially for those characteristics that relate to the attractiveness of the mur A-lines to insect pollinators.

8. Initiate a study of all aspects of field scale hybrid seed production using mur CMS A-lines, B-lines and R-lines.

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