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

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John Lawrence Sernyk

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ABSTRACT

SERNYK, JOHN LAWRENCE. Ph.D., The University of Manitoba, October 1982. Heterosis and cytoplasmic male sterility in summer rape (Brassica napus L.). Major Professor; Baldur R. Stefansson.

Heterosis and cytoplasmic male sterility were investigated in summer rape (Brassica napus L.) during three years of field trials (1979 to 1981).

Heterosis for seed yield of 40% over the conventional canola rape cultivar Regent was found in several hybrids with only a one or two day delay in maturity. Two of the high yielding hybrids (Marnoo X Regent and Karat X Regent) were of canola quality. The hybrids were very vigorous in growth. This vigor resulted in increased total dry matter production and higher apparent harvest indices for the hybrid genotypes. The vigor and increased seed yields of rape hybrids suggest that development of hybrids for commercial production could be justified.

The nap cytoplasmic male sterility (cms) system in B. napus was evaluated as a mechanism for pollination control in hybrid seed production. The male sterility in the initial winter rape lines obtained from Japan was not stable under local conditions but stable summer rape selections

were developed. The best selections gave complete pollination control under field conditions. The male sterile selections had narrow petals, short stamens, small cone-shaped anthers and low position of anther with respect to stigma typical of the nap cms system. A maintainer for these summer cms lines was developed using the male fertile cam cytoplasm of the Polish land race cultivar Bronowski. An estimated 8 nuclear male fertility restorer genes were found to be present in the canola cultivar Regent. Therefore this system is difficult to use in hybrid rape production.

The inheritance in B. napus of an "introgressed" white flower color derived from Raphanobrassica was investigated genetically and cytologically in view of the fact that the use of alien cms systems (ogu and mur systems) for pollination control will involve the incorporation of restorer genes from these alien species. The white flower color in this B. napus line had not been introgressed into the rape genome but was associated with a small radish (Raphanus sativus L.) chromosome. Thus the true breeding white flowered line was a disomic alien addition line with $2n=40$. The meiotic behavior of the additional chromosome as a univalent was studied. The chromosome was incorporated into about 25% of the gametes. However, strong certation effects resulted in varying degrees of transmission through the pollen as observed in monosomics of other crops. The

results suggest that introgression of alien traits may be difficult and that both genetic and cytological observations will be necessary to determine if introgression has taken place.

FOREWORD

This thesis has been prepared in manuscript style. The first two manuscripts have been submitted for publication to the Canadian Journal of Plant Science, the third manuscript is not being submitted for publication, and the fourth manuscript has been accepted for publication by the Canadian Journal of Genetics and Cytology.

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WHITE FLOWER COLOR IN RAPE (*BRASSICA NAPUS* L.) ASSOCIATED WITH A RADISH (*RAPHANUS SATIVUS* L.) CHROMOSOME

INTRODUCTION

During the last two decades, rape has developed from a minor to a major crop in western Canada. Annual rapeseed production in western Canada has averaged 2.5 million tonnes from 2.1 million hectares during the last three years (Statistics Canada).

The development of new cultivars with improved quality has been a major factor in the success of rape in Canada (Downey et al., 1975). These improvements in quality were of such a magnitude that in 1979 the Western Canadian Oilseed Crushers Association (now the Canola Crushers of Western Canada) adopted a new commodity name, canola rapeseed, to distinguish the new rapeseed and rapeseed products with low erucic acid content in the oil and low glucosinolate content in the meal from the old or common rapeseed.

Interest in the possibility of developing hybrid rape cultivars with substantially higher seed yield potential was stimulated by reports of cytoplasmic male sterility (Shiga and Baba, 1971, 1973; Thompson, 1972). Several reports (Schuster and Michael, 1976; Shiga, 1976; Morice, 1978; Buson, 1980; Guan, 1980; Hutcheson et al., 1981) indicate that seed yields of F1 hybrids of rape (Brassica napus L.

and Brassica campestris L.) may exceed those of the conventional cultivars by up to 40 to 60%.

In 1979, research was initiated at the University of Manitoba to investigate in detail the two major requirements for F1 hybrid rape production in western Canada. These two requirements are: (1) sufficient heterosis or hybrid vigor for seed yield in locally adapted hybrids to justify the additional costs involved in hybrid seed production and (2) a pollination control system for use in the field scale production of hybrid seed. Heterosis was investigated in hand-crossed, intervarietal hybrids of summer rape (B. napus) using top crosses between the Canadian canola cultivar Regent and a number of foreign summer rape cultivars. The nap cytoplasmic male sterility system (Shiga, 1980) was evaluated as a pollination control system.

The possibility of utilizing alien cytoplasms from the radish (Raphanus sativus L.) and the sand rocket (Diplotaxis muralis L.) as male sterile cytoplasms for hybrid rape production (Shiga, 1980) probably will require the transfer of male fertility restorer genes from these alien species into the B. napus genome. A study of a white flower color in B. napus introduced from R. sativus by way of a Raphanobrassica hybrid was initiated in 1979 to examine the genetics and cytological behavior of such an alien trait in the B. napus genome.

LITERATURE REVIEW

Heterosis

The term heterosis was first used by Shull (1914) to represent the special stimulus of heterozygosis on "cell-division, growth and other physiological activities of an organism". Shull (1948) defined heterosis as "the increase of size, yield, vigor, etc." resulting from the heterozygosis present in hybrids between divergent parents. From the crop production viewpoint, heterosis perhaps is best defined as the yield superiority of a hybrid over the best open-pollinated or inbred cultivar.

MacKey (1976) discussed the genetic mechanisms of heterosis. MacKey subdivides the genetic regulation of heterosis into:

1. genomic heterosis
    a) nonallelic heterosis
    b) allelic heterosis
        i) dominant heterosis
        ii) overdominant heterosis
2. plasmatic heterosis
3. non-heritable maternal heterosis.

The classic example of nonallelic heterosis is that of Powers' (1944) tomato fruit yield data. Powers found that

heterosis for fruit yield could occur as a result of intermediate reactions of the two nonallelic components of fruit yield, namely fruit number and fruit weight.

Allelic heterosis was explained by Bruce (1910) and Jones (1917) as a sheltering, inhibition, or masking of the effects of deleterious recessive alleles by dominant alleles at many loci. This theory of Jones usually is referred to as the "dominance theory of heterosis". Shull (1908, 1911), East (1908) and Hull (1945) explained allelic heterosis as a cooperation of alleles in a stimulatory, complementary or dossage-compensating way such that the heterozygote (Aa) is superior to both of the possible homozygotes (AA,aa). This theory usually is referred to as the "overdominance theory of heterosis".

The contribution of cytoplasmic factors to heterosis was first demonstrated by Hanson et al. (1960). They reported that the mitochondrial activity in seedlings of F1 hybrid corn (Zea mays L.) was correlated with the degree of heterosis expressed by the hybrid. This phenomenon has since been extensively investigated in plants (McDaniel and Sarkissian, 1966, 1968; McDaniel, 1972, 1974). Mitochondrial based heterosis arises from the polymorphism of mitochondria in cells of hybrid organisms and the complementation resulting from these mixed mitochondrial populations. This phenomenon is usually referred to as mitochondrial complementation. Mitochondrial

complementation also may be an explanation for the greater stability observed in hybrids exposed to environmental stress (MacKey, 1976).

The last mechanism which MacKey (1976) discussed is that of non-heritable maternal heterosis. This mechanism involves factors such as seed maturation, seed dormancy, seed size and endosperm characteristics which may influence the superiority of hybrid offspring.

MacKey (1976) also discussed the relative importance of these mechanisms. MacKey concluded that the dominance, overdominance, nonallelic and plasmatic regulatory systems are all important mechanisms of heterosis and that their relative importance will depend upon the reproductive system of the species, history of selection and the character concerned.

While several genetic mechanisms may explain heterosis, it is the level of heterosis available for exploitation in a plant species which will determine whether hybrids can be produced on a commercial basis. The level of heterosis for grain yield has been determined in most of the major crop species including corn (Sprague and Eberhart, 1977), wheat, Triticum aestivum L. (Nettevic, 1965), rice, Oryza sativa L. (Carahan et al., 1972), barley, Hordeum vulgare L. (Hayes and Foster, 1976), oats, Avena sativa L. (Hathcock and McDaniel, 1973), and soybeans, Glycine max (L.) Merr. (Weber et al., 1970). Levels of heterosis for grain yield from 40%

to more than 100% over the better parent or over conventional cultivars have been reported in these crops.

Heterosis has been investigated in rape (B. napus) in Europe, Japan and China. Schuster and Michael (1976) studied inbreeding depression and heterosis in winter rape (B. napus) to determine the potential for developing synthetics. In spite of the high rate of self-pollination of B. napus, they found inbreeding depression comparable to that in corn, a cross-pollinating species. They were, however, able to obtain some inbred lines which showed higher yields than those of the heterozygous parental cultivar. Heterosis for seed yield of 17% over standard winter rape cultivars was observed in about one-fifth of the F1 hybrids evaluated. A few of the hybrids exhibited heterosis for seed oil content over standard winter rape cultivars.

Shiga (1976) investigated heterosis in F1 hybrids made between Japanese winter rape (B. napus) cultivars. Shiga evaluated 62 such intervarietal hybrids between 1954 and 1967 and found half of the hybrids to be intermediate between the two parents in maturity, Sclerotinia disease resistance, height, length of inflorescence, total number of branches, and thousand seed weight. One-third to one-quarter of the hybrids showed heterosis over the desirable parent in length of inflorescence, total number of branches, number of primary branches, one liter seed weight

and thousand seed weight. The performance of 48 of the 62 hybrids was superior to that of the more desirable parent. Shiga observed significant heterosis for seed yield over the midparent value in the majority of the hybrids evaluated. Shiga classified the hybrids according to the origins of the parents as Chosenshu x Chosenshu, Chosenshu x European type cultivar, and European type cultivar x European type cultivar. Hybrids of the Chosenshu x European type cultivar showed the greatest levels of heterosis for seed yield (averaging approximately 40% over the midparent values).

Shiga (1976) also investigated heterosis in F1 hybrids between the Japanese cytoplasmic male sterile line and 131 Japanese winter rape cultivars. The seed yields of most of these F1 hybrids showed heterosis over the seed yields of the parental winter rape cultivars (averaging approximately 40% in the better hybrids). Heterosis for plant height also was observed in most of the hybrids. The majority of the hybrids was equal or superior to the parental cultivars in resistance to lodging, thousand seed weight and Sclerotinia disease resistance. Shiga noted that there was no relationship between the male fertility restoring ability of these lines and the level of heterosis observed in the hybrids.

Buson (1980) studied heterosis in an incomplete diallel experiment involving 25 winter rape (B. napus) inbred lines. A total of 130 F1 hybrids was evaluated, the combinations made being based on the consanguinity of the inbred lines.

The F1 hybrids and parents were grown in 4 row, 3 m long plots with 30 cm row spacing, using 3 replicates. Overall, hybrids showed 23% superiority over inbreds for seed yield but some of them reached 50% over the midparent value. Hybrid vigor also was noted in characters associated with vegetative growth (leaf area and plant height) and yield components (number of siliqua (pods) per plant and number of seeds per silique).

Buson (1980) also estimated general and specific combining ability variances and effects. She found that general combining ability was the most important component of the heterosis observed for seed yield, thus suggesting that additive gene action is the major contributor to seed yield in these hybrids (Griffing, 1956).

Guan (1980) studied heterosis using 8 intervarietal hybrids and 11 hybrids of male-sterile lines crossed with fertility restoring cultivars of rape (B. napus) in China. Guan found significant heterosis in the number of primary branches and number of siliqua per plant. Up to 60% heterosis for seed yield over the better parent was observed.

Guan (1980) also determined stomatal number per unit area of silique 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 of these

characteristics suggesting that the hybrids have a greater photosynthetic capacity as the basis for their vigorous growth and high yields.

To summarize, studies on heterosis in rape (B. napus) indicate that F1 hybrids have the potential to produce seed yields 40 to 60% greater than those of conventional rape cultivars. However, quality and agronomic performance are also major factors to be considered in the production of F1 hybrids and some of this heterosis may have to be sacrificed in order to meet these requirements.

Seed Yield, Biological Yield and Harvest Index

In addition to seed yield per se, biological yield and harvest index have been used as criteria for selection towards higher yields in cereals (Donald and Hamblin, 1976). Harvest index is seed yield expressed as a proportion or percent of the biological yield of a crop. Ideally, the biological yield used to compute harvest index should include all above and below ground structures, but for practical reasons usually only the above ground structures remaining at maturity (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. Apparent harvest index and harvest index have been shown to be positively correlated (r=0.97) in soybeans (Schapaugh and Wilcox, 1980).

Niciporovic (1956) emphasized that effective exploitation of photosynthesis to achieve maximum biological yield was the key to successful crop production. Donald (1962) suggested that in addition to greater photosynthesis and biological yield, plant breeders should also select for the ability to render a greater proportion of this biological yield as seed (i.e. a greater harvest index).

The interrelationships among seed yield, biological yield and harvest index were discussed by Donald and Hamblin (1976). They suggest that the situation where seed yield is positively correlated with harvest index and neither seed yield nor harvest index are related to biological yield is typical of cultivar trials where biological yield is fairly uniform across cultivars and seed yield and harvest index vary together. A positive correlation between seed yield and biological yield along with no relationship between seed yield or biological yield and harvest index may be typical of genotypes competing in mixtures such as in a segregating population. Positive correlations among seed yield, biological yield and harvest index is typical of the responses of a crop to water under suboptimal moisture conditions. A positive correlation between seed yield and biological yield and negative correlations of seed yield and biological yield with harvest index is typical of responses of a crop to nitrogen when water is abundant. Finally, a positive correlation between seed yield and harvest index

and negative correlations of both seed yield and harvest index with biological yield is typical of responses of a crop to nitrogen when water is deficient.

In rape (B. napus), seed yield, total dry matter production and apparent harvest index have been investigated by several researchers. Thurling (1974) investigated the growth and morphological characters associated with yield in winter rape (B. napus) grown in Australia. Total plant dry weight, seed yield and apparent harvest index were determined at maturity on an individual plant basis. Typical apparent harvest indices ranged from 16 to 23% depending upon seeding date. The correlation between seed yield and total plant dry weight was positive and highly significant (r=0.70), the correlation between seed yield and apparent harvest index was not significant (r=0.14), and the correlation between total plant dry weight and apparent harvest index was negative and highly significant (r=-0.54). A multiple regression of seed yield on total plant dry weight and apparent harvest index indicated that these two characteristics accounted for 87% of the variability observed in seed yield.

Campbell and Kondra (1978) investigated the relationships among growth patterns, yield components and seed yield of summer rape (B. napus) on an individual plant basis. They found seed yield was correlated positively with vegetative yield and with apparent harvest index in all three cultivars

tested. Vegetative yield was positively correlated with apparent harvest index in one of the cultivars but was uncorrelated in the other two cultivars. These results are suggestive of the response of a crop to water under suboptimal moisture conditions (Donald and Hamblin, 1976) which could well be the situation for summer rape grown in western Canada.

Cytoplasmic Male Sterility

Cytoplasmic male sterility (cms) refers to the condition of pollen sterility controlled by a heritable, cytoplasmic factor. Pring *et al.* (1977) have found evidence to suggest that this cytoplasmic factor is a gene on a mitochondrial chromosome. Male sterility due to a cytoplasmic factor usually can be overcome by nuclear genes referred to as male fertility restorers.

The classic example of cms is in corn (Rhoades, 1933; Duvick, 1965) but cms has been identified and utilized for hybrid seed production (Duvick, 1959) in a number of crops. These include onion, *Allium cepa* L. (Jones and Clarke, 1943), sorghum, *Sorghum bicolor* (L.) Moench (Stephens and Holland, 1954), rice (Shinjyo and Omura, 1966; Shinjyo, 1972) and sunflowers, *Helianthus annuus* L. (Leclercq, 1969; Whelan, 1980).

Care must be exercised with the use of cms systems to avoid disasters such as the southern corn leaf blight

(Helminthosporium maydis) epidemic which occurred in the U.S. in 1970. This epidemic resulted from the widespread use of the Texas male sterile cytoplasm in 90% of the U.S. corn hybrids and an association between this cytoplasm and susceptibility to southern corn leaf blight (Ullstrup, 1977). This disaster greatly reduced the use of cms in corn but recent trends have been towards increased use of other male sterile cytoplasms in hybrid seed production due to the high labor costs of detasseling. In 1979, approximately 20% of the U.S. corn hybrids were produced using cms (Zuber and Darrah, 1980).

Cms in rape (B. napus) was first reported by Shiga and Baba (1971,1973) and Thompson (1972). Shiga and Baba found male sterile plants in the progenies of a cross between the Japanese oil-seed winter rape cultivars Chisaya-natane and Hokuriku 23. Thompson found male sterile plants in the F2 generations of crosses with the Polish land race summer rape cultivar Bronowski as the male parent. In both cases, reciprocal crosses indicated that this male sterility was cytoplasmically inherited.

Shiga et al. (1976) observed that the cms line from Thompson was only partially male sterile under the conditions in Japan. They observed that maintainers and restorers for their cms line were also maintainers and restorers for the cms line from Thompson. This suggests that the two male sterile cytoplasms were similar if not the

same. Shiga (1980) classified this male sterile cytoplasm as the nap cytoplasm.

Shiga (1976) and Bartkowiak-Broda et al. (1979) studied the floral morphology of these nap cms lines. The male sterile plants had narrow rugose petals, short stamens, small cone-shaped anthers without pollen, and a low position of anther with respect to stigma. Shiga (1976) found partially male sterile plants to possess floral characteristics intermediate between those of male sterile and normal male fertile plants. Thus, based on floral morphology, Shiga was able to classify plants as male sterile, partially male sterile and male fertile.

Shiga (1976) studied anther development of male sterile and partially male sterile plants using cytohistological techniques. He estimated that the breakdown of anther development occurred between the stages of carpel differentiation III and male archesporial cell differentiation I. No anther loculi, pollen mother cells (PMCs) or anther tapeta were found in the anthers of the male sterile plants. In partially male sterile plants, male archesporial cells were produced and these occasionally developed into anther loculi. These loculi produced PMCs and anther tapeta and the PMCs underwent meiotic divisions to produce pollen.

Shiga (1976) also found the male sterile plants to be shorter and to have a somewhat lower seed fertility than their male fertile counterparts.

Thompson (1972) and Shiga (1976) both found the male sterility due to the nap cytoplasm to be unstable. It had a tendancy to break down at higher temperatures and during the later stages of flowering. Thompson concluded that this system would not be suitable for pollination control because of this instability but Shiga suggested that the stability of the male sterility in the nap system could be improved by selection. Shiga also pointed out that studies had shown that a population made up of F1 seed mixed with as much as 25 to 50% of parental seed produced yields comparable to a population grown from pure F1 seed under high density planting (Murakami et al., 1969; Shiga et al., 1970). Based on these findings, Shiga suggested that complete control of pollination was not necessary and some breakdown of the male sterility could be tolerated. Shiga also suggested that this instability could even be advantageous in that it allowed maintenance of the male sterile lines by self-pollination using the pollen which was formed rarely on the male sterile plants.

When testing the male sterile lines under field conditions, Shiga (1976) found about 97 to 98% outcrossing to occur. The considerable degree of male sterility and low position of anther with respect to stigma undoubtedly were responsible for the high level of outcrossing observed.

The genetics of fertility restoration in the nap cms system was also studied by Shiga. Shiga studied a number of

Japanese (Shiga, 1976) and European (Shiga *et al.*, 1978) winter rape cultivars. Shiga estimated that there are 3 or 4 loci involved in male fertility restoration in most of the Japanese and European cultivars studied. Male fertility restoration was controlled by dominant or partially dominant alleles at these loci. Shiga also classified the cytoplasms of these cultivars and found that the majority of the Japanese and European cultivars studied possessed the *nap* cytoplasm along with 3 or 4 male fertility restorer genes. A smaller number of cultivars (e.g. Hokuriku 23 and Bronowski) possessed a male fertile cytoplasm and few (only 1 or 2) if any male fertility restorer genes. Shiga (1980) suggests that this male fertile cytoplasm originated from *B. campestris* and thus classified it as the *cam* cytoplasm. Because *Brassica oleraceae* L. is the other parent of the amphidiploid, *B. napus*, the male sterile *nap* cytoplasm most likely originated from that species.

Shiga (1976) found strong correlations between floral characteristics (petal width, anther length and position of anther with respect to stigma) and the degree of male sterility (determined by seed set on bagging) in the *nap* cms system. Using petal width and position of anther with respect to stigma, Shiga was able to derive a fertility index equation which could be used to classify the male fertility of plants in the *nap* cytoplasm.

A second source of cytoplasmic male sterility in rape was developed by backcrossing the genome of B. napus into a cytoplasm from a male sterile radish (R. sativus) (Ogura, 1968; Bannerot et al., 1974; Bartkowiak-Broda et al., 1979). Shiga (1980) classified this male sterile cytoplasm as the ogu cytoplasm after its discoverer.

Bartkowiak-Broda et al. (1979) studied the morphology and nature of this cms system in rape (B. napus). Petal width was almost normal in the ogu cms lines and atrophy of the stamens and complete absence of pollen grains was always observed. Female fertility was normal in the cms lines. Cytohistological studies indicated that meiosis and microspore production occurred normally in the ogu cms lines but then the microspores degenerated, probably due to a breakdown of the tapetum.

Heyn (1979) and Rouselle (1979) found that the male sterile lines of rape in the ogu cytoplasm were chlorophyll deficient at cooler temperatures. Both Heyn and Rouselle were unable to find male fertility restorers for the ogu cms system among the lines of rape (B. napus) tested. Heyn attempted to obtain male fertility restorers for the ogu cms system from the radish (R. sativus) through Raphanobrassica. His results suggested that male fertility restoration was controlled by two loci with complementary dominant gene action. Heyn also suggested that one of the male fertility restorer genes was linked to one of two genes for white

flower color in the radish. He thus suggested that dominant white flower color could be used as a marker for the presence of that restorer gene.

A third source of cms in rape was obtained by backcrossing B. napus into the cytoplasm of D. muralis, the sand rocket. Shiga (1980) classified this cytoplasm as the mur cytoplasm. Shiga suggested that male fertility restorers for this cms system are not present in B. napus and thus would have to be introduced from D. muralis. The mur cms system has not been extensively studied in B. napus but a mur cms system in B. campestris has been developed in Japan (Hinata and Konno, 1979). This mur cms system in B. campestris has a strong male sterility and only a single male fertility restorer. The plants are deep green and vigorous in growth habit.

To summarize, there are three main cms systems currently available in rape (B. napus) for possible use in F1 hybrid seed production. Of these, the mur cms system seems the best suited system in that male sterility is complete and restoration is simple (1 locus). The functioning of B. napus in this cytoplasm still needs to be tested and the introgression of the D. muralis male fertility restorer gene into the B. napus genome has to be accomplished. The nap cms system has unstable male sterility and too many (3 or 4) male fertility restorer loci, although it is native to B. napus and thus well tested. Male sterility in the ogu cms

system is complete but there are problems due to incompatibility of *B. napus* with this cytoplasm. Also male fertility restorer genes for the *ogu* cms system will have to be successfully incorporated into the *B. napus* genome from *R. sativus*.

HETEROSIS IN SUMMER RAPE (*BRASSICA NAPUS* L.)

J. L. Sernyk and B. R. Stefansson

Department of Plant Science

University of Manitoba

WINNIPEG, Manitoba, Canada R3T 2N2

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Abstract

The degree of heterosis for seed yield in F1 hybrids of summer rape (*Brassica napus* L.) was examined in replicated yield trials during two years (1980 and 1981) using intervarietal hybrids produced by manual crossing. The seed yields from the F1 hybrids of crosses between Marnoo and Regent, and Karat and Regent exceeded those of Regent by 38 and 43 %, respectively. With the possible exception of maturity, which was one day later than Regent, the agronomic and quality characteristics of these hybrids were within the ranges acceptable in commercial rape cultivars. However, the successful development of hybrid rape cultivars will depend upon the development of a suitable genetic or chemical (male gametocide) pollination control system.

Introduction

During the last two decades, rape has developed from a minor to a major crop in western Canada. Annual rapeseed production in western Canada has averaged 2.5 million tonnes from 2.1 million hectares during the last three years (Statistics Canada).

The development of new cultivars with improved quality has been a major factor in the success of rape in Canada. The improvements in quality were of such a magnitude that in 1979 the Western Canadian Oilseed Crushers Association (now the Canola Crushers of Western Canada) adopted a new commodity name, canola rapeseed, to distinguish the new rapeseed and rapeseed products with low erucic acid content in the oil and low glucosinolate content in the meal from the old or common rapeseed.

Several studies (Schuster and Michael, 1976; Shiga, 1976; Morice, 1978; Buson, 1980; Guan, 1980; Hutcheson *et al.*, 1981) report that seed yields of F1 hybrids of rape (*Brassica napus* L. and *Brassica campestris* L.) may exceed those of their parents by 40 to 60 %. This plus reports of cytoplasmic male sterility (Shiga and Baba , 1971 , 1973; Thompson , 1972) has stimulated interest in the development of hybrid rape cultivars. The experiments described in this paper were designed to determine the level of heterosis for seed yield in F1 hybrids of *B. napus* grown in western Canada.

Materials and Methods

In 1980, seven cultivars of *B. napus* and in 1981, four cultivars were reciprocally topcrossed to the Canadian canola cultivar 'Regent' (Table 1). Cultivars from different countries were chosen because heterosis can be expected in hybrids produced from divergent parents. Two of the cultivars used in 1981, Marnoo and Karat, were added because they are canola quality cultivars from foreign countries. A topcross to the cultivar Regent was used to permit comparison of all hybrids to a common, widely grown, locally adapted parent.

F1 hybrid seed was produced in the greenhouse during the fall and winter months. Crossing was accomplished by emasculation and bud pollination. The crossed racemes were bagged to prevent contamination. At least 40 pairs of plants were used to produce the seed of each F1 hybrid to ensure an adequate sampling of the variability within each of the cultivars. The parental cultivars were maintained by bagging racemes (selfing) on the same plants used to produce the F1 hybrid seed. To test for inbreeding depression in the topcross parent Regent, Regent Breeders seed from the same source as the seed used for crossing was included in the yield trials. The harvested seed was cleaned on a laboratory model of a spiral seed cleaner to eliminate chaff and shrivelled, poorly developed seed. Germination percentages and thousand seed weights were determined for F1 hybrid, maintained parent, and Regent Breeders seed.

A randomized complete block design (RCBD) with 3 blocks was used in both years. Each block included two Regent Breeders seed plots, two plots sown with Regent self-pollinated seed and one plot of each of the other cultivars, the F1 hybrids and the reciprocal F1 hybrids. The yield trials were carried out on the University of Manitoba campus field plots (Winnipeg , Canada). The soil type was a Fort Garry clay with poor subsurface drainage. Plot areas were summerfallowed the season prior to planting. The herbicide Treflan was applied in granular form at the recommended rate (28 kg/ha) during seed bed preparation in the fall.

In the spring, soil samples were taken to determine soil fertility. Seeding in 1980 was on May 21 and in 1981 was on May 20 and June 8. The June 8 seeding date in 1981 was required because hybrid seed was not available prior to that date. Seeding to adequate moisture at a 2.5 to 4.0 cm depth was accomplished with a 4 row belt seeder and packer. The plot size in 1980 was 1.2 m X 4.6 m and in 1981, 1.2 m X 5.8 m with a 30 cm spacing between rows in both years. Seeding rates were adjusted based on germination percentage and seed size to give approximately a 2.5 cm spacing between plants in the row. This resulted in a seeding rate for Regent of 4 kg/ha. Furadan 10G was applied with the seed at about 5 times the recommended rate of 2.8 kg/ha for both flea beetle and cabbage root maggot control. Fertilizer (11-48-0) was

applied with the seed at a rate of 15 kg P_2O_5 / ha which also supplied 3.4 kg N / ha.

In 1980, due to low soil moisture and warm spring temperatures, 10 cm of irrigation was applied June 19 to 22. In 1981, no irrigation was applied. Plots were sprayed occasionally with recommended insecticides to control flea beetles throughout the growing season in both years.

Agronomic characteristics, including days to emergence, days to first flowering, days to maturity, height at maturity, and lodging at maturity, were recorded for all plots.

At maturity, the center 3 m of the middle 2 rows of each plot was hand harvested at a stage when approximately 50% of the seed in that plot had started to turn brown or black in color. The entire above-ground plant material of the harvested plot areas was placed in burlap sacks and air-dried for several weeks before threshing. Seed yields for the harvested area of each plot were determined and samples of seed were taken for moisture determination. The moisture free weights of seed from each plot were calculated and converted to seed yields in kg/ha. Thousand seed weight was determined using oven-dried seed samples.

Seed samples from each plot were analyzed for oil and protein content. Oil percent was determined by the Nuclear Magnetic Resonance technique (Robertson and Morrison , 1979) using 25 g oven-dried seed samples. Protein percent was

determined using the standard Kjeldahl procedure, but with titanium dioxide as the catalyst in the digestion (Williams , 1973).

The data from both years were entered into the Amdahl 470/V7 computer system on the University of Manitoba campus using MANTES (Ferch et al. , 1978). The SAS package (Helwig and Council , 1979) was used to analyze the data. The data were analyzed using the standard analysis of variance for a RCBD. Single degree of freedom contrasts were used to compare the mean values for Regent with those for the F1 hybrids. Pearson product-moment correlations between yield and other characteristics within the topcross parent Regent and within the three high yielding hybrids (Gullivar X Regent, Marnoo X Regent and Karat X Regent) as a group were determined.

Results

Agronomic, yield and quality data for the trials are presented in Table 2. Relative seed yields of the trials are best understood using the common entry, Regent. The seed yield of Regent was 2198 kg/ha and 1529 kg/ha in 1980 and 1981 (early planting), respectively. The higher yields in 1980 likely resulted from the supplemental irrigation in June. In 1981, a hot dry period in July adversely affected yields.

Soil test data indicated that the fertility of the plot areas in both years was adequate for the production of a 1960 kg/ha rape crop. The 11-48-0 fertilizer applied with the seed was supplementary to this.

Data for the hybrids involving the three Japanese cultivars were not included as these hybrids and their parents matured too late to permit evaluation in the short season in western Canada. Because the results from reciprocal crosses did not differ significantly at the 5 % level, the data were pooled. Similarly, the data from Regent maintained by self pollination did not differ significantly from data from Regent Breeders seed, and therefore the two sets of data were pooled as data for Regent.

Agronomic Characteristics

The number of days from seeding to emergence for the F1 hybrids was equal to or slightly less (one or two days) than the days to emergence for Regent (Table 2).

The high yielding F1 hybrids (Gullivar X Regent , Marnoo X Regent , and Karat X Regent) were easily distinguishable from their parents on the basis of their vegetative vigor. The F1 plants developed and covered the plot area more rapidly than their parents. This vegetative vigor apparently also gave the hybrids greater tolerance to flea beetles.

Days to first flowering indicated a partial dominance of earliness in all hybrids (Table 2). The three high yielding hybrids flowered only one day later than Regent.

Days to maturity also suggested a partial dominance of earliness in all hybrids, the three high yielding hybrids maturing only one or two days later than Regent (Table 2).

Tall stature was partially dominant except in the late seeded trial in 1981, where overdominance of tall stature was observed (Table 2). In this late seeded trial, flowering was prematurely terminated by fall weather conditions in the two late (tall) parents giving the F1's an apparent overdominance for tall stature. The three high yielding hybrids were only 8 to 12 % taller than Regent.

The hybrids were equal to or better than Regent in their resistance to lodging (Table 2). In 1981, the entire May 20th trial was severely lodged due to strong winds on July 30th.

Seed Yield

Significant heterosis for seed yield (Tables 2 and 3) was observed in the Gullivar X Regent, Marnoo X Regent and Karat X Regent hybrids (38 to 43 % over Regent). Other hybrids also showed some heterosis: Schuster 75-01 X Regent (15 to 18 % over Regent) and Kosa X Regent (7 % over Regent). The Bronowski X Regent hybrid showed no heterosis for seed yield over Regent.

Seed yield was positively correlated (Table 4) with days to first flowering, days to maturity and height at maturity both within Regent and within the three high yielding hybrids, Gullivar X Regent, Marnoo X Regent and Karat X Regent. There was no significant correlation between seed yield and lodging although the negative sign suggested that higher yield was associated with less lodging. Thousand seed weight was not correlated with seed yield within Regent but was positively correlated with seed yield within the three high yielding hybrids. Seed oil content was not correlated with seed yield but seed protein content was negatively correlated with seed yield, although the correlation was statistically significant only for Regent.

Quality Characteristics

Two of the three high yielding hybrids, Gullivar X Regent and Marnoo X Regent, sown under the early planting dates did not differ significanlty from Regent in thousand seed weight (Table 2). The third of the high yielding hybrids, Karat X Regent, sown late in 1981 was significantly lower in thousand seed weight than Regent. The Schuster 76-01 X Regent hybrid was significantly lower in thousand seed weight than Regent in both years. The thousand seed weight of the Kosa X Regent hybrid was equivalent to that of Regent and the Bronowski X Regent hybrid had a significantly lower thousand seed weight than Regent. The general trend was

towards a thousand seed weight intermediate between the thousand seed weights of the two parents.

The seed oil content of the three high yielding hybrids, Gullivar X Regent, Marnoo X Regent and Karat X Regent, was comparable to that of Regent (Table 2). The Schuster 75-01 X Regent hybrid was significantly higher than Regent in oil content in the early planting date in 1980 but not in the late planting date in 1981. The Bronowski X Regent hybrid was also higher in oil than Regent and the Kosa X Regent hybrid was not different from Regent in oil content.

The protein content of the seed from the F1 hybrids was negatively associated with oil content (Table 2). The protein content of the seed from the three high yielding hybrids was within 1 % of that from Regent.

Discussion

The degree of heterosis consistently observed in F1 hybrids during the two years of testing demonstrates that it is possible to develop hybrid cultivars of *B. napus* with seed yields 40 % higher than those of Regent. The better hybrids were very vigorous and thus emerged and developed a crop canopy more rapidly than Regent. Early flowering, early maturity and tall stature were partially dominant. In spite of the yield advantage, resistance to lodging was equal to or better than that for Regent. Thousand seed weight, seed oil content and seed protein content were comparable to those for Regent.

The canola quality standards in Canada and the known inheritance of the characteristics involved require that both parents must be low in the erucic acid content of the oil and in the glucosinolate content of the seed so that the seed from the F1 hybrid will be of acceptable quality (Jonsson , 1977; Kondra and Stefansson , 1970). Thus the two double-low (low erucic acid, low glucosinolate) hybrid combinations, Marnoo X Regent and Karat X Regent, are of particular interest. They exhibit respectively, 38 % and 43 % heterosis for seed yield over Regent, are of canola quality, and are only 1 day later maturing than Regent. Evaluation of these hybrids along with the Marnoo X Karat hybrid will be continued. The Gullivar X Regent hybrid also showed significant heterosis (40 to 43 % over Regent), but was not of canola quality due to the high glucosinolate genotype of the Gullivar parent.

Conclusion

The level of heterosis for seed yield (approximately 40 %) obtained for certain F1 hybrids of rape (B. napus) appears to be sufficient to justify the added costs involved in hybrid seed production. However, the development of hybrid cultivars for commercial production still depends upon the development of suitable genetic (Shiga , 1980) or chemical (Dotlacil and Apltauerova , 1978; Van Der Meer and Van Dam , 1979) systems for pollination control.

Acknowledgements

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TABLE 1. Cultivars of *B. napus* used in the hybrid rape yield trials, their countries of origin, and years in the trials.

Cultivar	Country of origin	Years in trials
Regent#	Canada	1980-81
Asahi-natane	Japan	1980
Chisaya-natane	Japan	1980
Norin 16	Japan	1980
Kosa	Germany	1980
Bronowski	Poland	1980
Gullivar	Sweden	1980-81
Schuster 75-01	Germany	1980-81
Karat#	Sweden	1981
Marnoo#	Australia	1981

Canola quality cultivars.

TABLE 2. Agronomic, yield and quality data for the hybrid rape (*B. napus*) yield trials.

Seeding date	Cultivar/ Hybrid	Days to emergence	Days# to flowering	Days# to maturity	Height (cm)	Lodging (1-5)^	Seed yield (kg/ha)	Thousand seed weight (gm)	Oil$ (%)	Protein$ (%)
May 21,1980	Regent	14	37	95	116	2.2	2198	3.25	44.3	27.9
	(GXR)F1	13	38	97**	131**	1.8	3072**	3.30	44.1	27.4
	Gullivar	14	42	101	134	1.7	2272	3.20	43.4	27.1
	(SXR)F1	14	40**	98**	133**	1.8	2525	2.78**	45.8**	25.4**
	Schuster	15	46	102	140	2.7	1899	2.47	43.2	25.7
	(KXR)F1	14	38	95	129**	1.8	2355	3.20	43.5	26.6**
	Kosa	14	41	96	137	2.3	2198	3.00	41.7	26.3
	(BXR)F1	15	40**	97**	133**	1.0**	2160	2.75**	45.7**	26.7**
	Bronowski	16	48	100	140	1.3	1512	2.00	44.9	25.9
	C V+ (%)	14.0	3.1	1.9	3.8	23.9	15.6	6.4	1.7	1.9
May 20,1981	Regent	12	36	81	104	3.0	1529	3.26	45.1	27.6
	(GXR)F1	10**	37**	83**	113**	3.0	2187**	3.17	44.3*	26.8*
	Gullivar	11	40	85	115	3.0	1130	3.45	39.5	31.1
	(MXR)F1	11*	37**	82*	111**	3.0	2106**	3.18	45.9*	26.0**
	Marnoo	11	41	85	112	3.0	1484	3.15	42.9	29.0
	C V (%)	5.9	1.6	1.0	4.0	0.0	15.6	3.4	1.5	2.2
June 8,1981	Regent	5	35	83	97	2.8	1082	3.35	43.8	29.1
	(KXR)F1	5	36	84	109**	2.3	1545**	2.99**	44.0	28.7
	Karat	7	41	88	91	1.0	908	3.09	42.4	30.7
	(SXR)F1	5	38**	86**	109**	2.8	1277	2.99**	43.9	27.2**
	Schuster	5	50	99	98	1.3	978	2.81	42.9	25.7
	C V (%)	4.6	2.8	1.1	5.7	25.9	20.9	4.6	1.5	2.8

\# From emergence.
^ 1 = no lodging; 5 = completely lodged.
$ Percent of seed dry weight.
\+ Coefficient of variation.
* F1 values significantly different from values for Regent.
** F1 values highly significantly different from values for Regent.

TABLE 3. Heterosis for seed yield in rape (*B. napus*) hybrids.

Hybrid	Year	Heterosis for seed yield		
		% of Regent	% of midparent	% of other parent
Bronowski X Regent	1980	-2	+16	+43
Kosa X Regent	1980	+7	+7	+7
Gullivar X Regent	1980	+40	+37	+35
	1981	+43	+64	+94
Schuster X Regent	1980	+15	+23	+33
	1981	+18	+24	+31
Marnoo X Regent	1981	+38	+40	+42
Karat X Regent	1981	+43	+55	+70

Table 4. Correlations between yield and other characters within the topcross parent Regent and within the three hybrids showing significant heterosis (Gullivar X Regent, Marnoo X Regent and Karat X Regent) as a group.

	Correlation coefficient	
	Regent (N=36)	Hybrids (N=24)
Character	Yield	Yield
Days to flowering	.35*	.56**
Days to maturity	.66**	.76**
Height	.74**	.82**
Lodging	-.26	-.30
Thousand seed weight	.17	.70**
Oil (%)	.16	-.04
Protein (%)	-.41*	-.23

* Significant.
** Highly significant.

INTERRELATIONS AMONG SEED YIELD, DRY MATTER PRODUCTION AND APPARENT HARVEST INDEX IN SUMMER RAPE (*BRASSICA NAPUS* L.) HYBRIDS

J. L. Sernyk and B. R. Stefansson

Department of Plant Science

University of Manitoba

WINNIPEG, Manitoba, Canada R3T 2N2

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Abstract

F1 hybrids of summer rape (*Brassica napus* L.) exhibited significant heterosis for seed yield, total dry matter yield and apparent harvest index. Correlations among these characteristics were positive and highly significant. The heterosis observed for seed yield, total dry matter yield and apparent harvest index occurred without any lengthening in the relative duration of the reproductive growth period. The hybrids were vigorous throughout both the vegetative and reproductive growth periods.

Introduction

In addition to seed yield per se, total biological yield and harvest index have been used as criteria for selection towards higher seed yield (Donald and Hamblin , 1976). Harvest index is seed yield expressed as a proportion or percent of the total biological yield of a crop. Ideally the total biological yield used to compute harvest index should include all above and below ground structures, but for practical reasons usually includes only the above ground structures remaining at maturity (seed plus straw). 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 yield at maturity.

Seed yield, total dry matter yield, and apparent harvest index have been previously examined in conventional rape (Brassica napus L.) cultivars (Thurling , 1974; Campbell and Kondra , 1978). This paper reports a study of these characteristics in F1 hybrids between cultivars of B. napus.

Materials and Methods

First generation hybrids from crosses between the Canadian canola cultivar Regent and cultivars from other countries were evaluated in replicated yield trials for two years (1980 and 1981) using randomized complete block designs (RCBD) (Sernyk and Stefansson , 1982). Each block included two Regent Breeders seed plots, two plots sown with

Regent self-pollinated seed and one plot of each of the other cultivars, the F1 hybrids and the reciprocal F1 hybrids. Seed yield and total harvested dry matter (seed plus straw) were recorded for each plot. Apparent harvest index was calculated by dividing the weight of the seed plus straw into the weight of the seed and multiplying by 100. The relative duration of the reproductive growth stage (days from flowering to maturity divided by days from emergence to maturity multiplied by 100) also was determined for all plots.

The data were analyzed using the standard analysis of variance for a RCBD. Single degree of freedom contrasts were used to compare mean values for Regent with those for the F1 hybrids. Pearson product-moment correlations were determined (1) within the topcross parent Regent, (2) within the three high yielding hybrids (Gullivar X Regent, Marnoo X Regent and Karat X Regent) as a group, and (3) using mean values for Regent and all hybrids.

Results

Significant heterosis for seed yield (Table 1) was observed in the Gullivar X Regent, Marnoo X Regent, and Karat X Regent hybrids (38 to 43 % over Regent). The Schuster 75-01 X Regent hybrid and the Kosa X Regent hybrid also showed some heterosis for seed yield (16 % and 7 % over Regent, respectively), but the Bronowski X Regent hybrid did not.

Heterosis for total dry matter yield was significant in the three high yielding hybrids, Gullivar X Regent, Marnoo X Regent, and Karat X Regent (Table 1). These three hybrids demonstrated 25 to 29 % heterosis for total dry matter yield over Regent. The other three hybrids which exhibited little or no heterosis for seed yield also showed a low level of heterosis for total dry matter production (8 to 12 % over Regent).

Heterosis for apparent harvest index of 9 to 14 % over Regent also was observed in the three high yielding hybrids (Table 1). Heterosis for seed yield was proportionately greater than heterosis for total dry matter yield in these hybrids.

Correlations among seed yield, total dry matter yield and apparent harvest index were positive and highly significant (Table 2).

The relative duration of the reproductive growth stage in the three high yielding hybrids was equivalent to or less than that for Regent (Table 1). The relative duration of the reproductive growth stage in the other three hybrids was less than that for Regent.

Discussion

The three high yielding hybrids of B. napus, Gullivar X Regent, Marnoo X Regent, and Karat X Regent, were very vigorous in vegetative growth compared to the parent cultivars (Sernyk and Stefansson , 1982). This vigor also was evident in the total dry matter production of these hybrids which was 25 to 29 % greater than that for Regent. The heterosis observed for apparent harvest index indicates that the hybrids also were able to convert a greater proportion of this increased total dry matter yield to seed production.

Positive correlations among seed yield, total dry matter yield, and harvest index are typical of the responses of a crop to water when grown under suboptimal moisture conditions (Donald and Hamblin , 1976). Thus the highly significant, positive correlations observed among seed yield, total dry matter yield, and apparent harvest index (Table 2) indicate that rape (B. napus), in western Canada responds significantly to water and that the hybrids are superior to Regent in their ability to utilize soil water resources. Guan's (1980) research, in which he found that hybrids of B. napus were superior to parental lines in exuding water rate, a measure of water translocation, also supports this conclusion.

The observed increases in seed yield, total dry matter yield, and apparent harvest index of the better hybrids

occurred without any significant lengthening of the reproductive growth stage. This indicates that the vigor observed in the hybrids prior to flowering continued throughout the reproductive growth period. Although root development was not studied, the vigor in top growth and the superior ability of the hybrids to exploit soil water resources would suggest that the hybrids also possessed a vigorous root system.

Conclusion

Heterosis for seed yield, total dry matter production and apparent harvest index was observed in rape (*B. napus*) hybrids. Heterosis for seed yield was proportionately greater than heterosis for total dry matter production. Correlations among seed yield, total dry matter production and apparent harvest index were positive and highly significant.

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TABLE 1. Yield, total dry matter, harvest index and reproductive growth period data for the hybrid rape (B. napus) yield trials.

Seeding date	Cultivar/ Hybrid	Seed yield (kg/ha)	Total dry matter yield (kg/ha)	Apparent harvest index (%)	Reproductive growth period (%)
May 21,1980	Regent	2198	7691	28.4	61.2
	(GXR)F1	3072**	9952**	30.9*	61.0
	Gullivar	2272	8751	26.2	58.1
	(SXR)F1	2525	8991*	28.1	58.6**
	Schuster	1899	8367	22.4	54.7
	(KXR)F1	2355	8317	28.2	60.1
	Kosa	2198	7701	28.5	56.8
	(BXR)F1	2160	8476	25.4*	58.2**
	Bronowski	1512	8620	18.0	52.0
	C V# (%)	15.6	11.4	8.1	2.1
May 20,1981	Regent	1529	6156	24.8	56.0
	(GXR)F1	2187**	7872**	27.8*	55.3*
	Gullivar	1130	6491	17.4	53.0
	(MXR)F1	2106**	7706**	27.2*	54.9**
	Marnoo	1484	6702	22.2	51.2
	C V (%)	15.6	9.3	8.9	0.9
June 8,1981	Regent	1082	5064	21.0	57.5
	(KXR)F1	1545**	6512**	23.9	57.6
	Karat	908	6273	14.7	53.0
	(SXR)F1	1277	5661	22.5	56.0**
	Schuster	978	6569	15.0	50.1
	C V (%)	20.9	14.7	16.3	1.7

\# Coefficient of variation.
* F1 values significantly different from values for Regent.
** F1 values highly significantly different from values for Regent.

TABLE 2. Correlations among seed yield, total dry matter yield and apparent harvest index.

	Total dry matter yield	Apparent harvest index
Seed yield	0.97**#	0.90**
	0.94**^	0.85**
	0.99**$	0.96**
Total dry matter yield		0.79**
		0.63**
		0.92**

Within the topcross parent Regent (N=36).
^ Within the three high yielding hybrids (Gullivar X Regent, Marnoo X Regent and Karat X Regent) as a group (N=24).
$ Among mean values for Regent and all hybrids (N=11).
** Highly significant.

EVALUATION OF THE NAP CYTOPLASMIC MALE STERILITY SYSTEM IN RAPE (*BRASSICA NAPUS* L.)

J. L. Sernyk and B. R. Stefansson

Department of Plant Science

University of Manitoba

WINNIPEG, Manitoba, Canada R3T 2N2

Abstract

Evaluation of the *nap* cytoplasmic male sterility (cms) system in a Japanese line of *Brassica napus* L. indicated that this line only partially male sterile under local conditions. A number of male sterile summer selections with greatly improved male sterility were developed from the filial generations of a cross between this line and the Canadian *B. napus* canola cultivar Regent. Some selections gave complete pollination control under field conditions. The male sterile selections had narrow petals, short stamens, small cone-shaped anthers and low position of anther with respect to stigma typical of the *nap* cms system. A maintainer for these summer cms lines was developed by backcrossing the male sterile selections (using pollen ocassionally produced in small quantities on these selections) into the male fertile *cam* cytoplasm of Bronowski, a Polish land race cultivar. The Canadian canola cultivar Regent was found to possess the *nap* cytoplasm and 5 complete and 3 partial dominant male fertility restorer genes. The large number of restorer genes in the majority of *B. napus* cultivars would make it difficult to use the *nap* cms system in hybrid rape production.

Introduction

The possibility of hybrid rape production is dependent upon two main requirements. The first is sufficient heterosis or hybrid vigor to make production of F1 hybrids economical and the second is a pollination control system suitable for hybrid seed production on a commercial scale.

Heterosis for seed yield of 40 to 60 % has been demonstrated in both species of rape, Brassica napus L. and Brassica campestris L. (Schuster and Michael, 1976; Shiga, 1976; Morice, 1978; Buson, 1980; Guan, 1980; Hutcheson et al., 1981; Sernyk and Stefansson, 1982). These results indicate that the first requirement is satisfied.

The second requirement, that of pollination control, has also been investigated. There are a number of mechanisms which are available for this purpose including sporophytic self-incompatibitity (Hinata and Nishio, 1980), nuclear and cytoplasmic male sterilities (Shiga, 1980) and male gametocides (Van Der Meer and Van Dam, 1979). Of the genetic mechanisms, cytoplasmic male sterility (cms) is probably the easiest to use and the most widely studied.

In rape (B. napus), there are three cms systems currently being evaluated, the nap, the ogu, and the mur cms systems (Shiga, 1980). Of these, the nap cms system is the only one occurring naturally in B. napus. The nap cms system was first reported by Shiga and Baba (1971,1973) and Thompson (1972). It is characterized by narrow petals, short

stamens, small cone-shaped anthers and low position of anther with respect to stigma (Shiga, 1976; Bartkowiak-Broda *et al.*, 1979). It is a temperature sensitive system, the male sterility breaking down at higher temperatures. Most cultivars of *B. napus* possess the *nap* cytoplasm and a large number of restorer genes (Shiga, 1980). The *nap* cytoplasm in all likelihood was derived from *Brassica oleraceae* L. Some *B. napus* and all *B. campestris* cultivars apparently possess the male fertile *cam* cytoplasm and few or no restorer genes (Shiga, 1980). The presence of the male fertile *cam* cytoplasm permits development of maintainer lines for the *nap* cms system.

Studies of the *nap* cms system in *B. napus* were carried out from 1979 to 1981 to: (1) evaluate the *nap* cms system under local field conditions, (2) develop a summer habit *nap* cms line which would be stable under local field conditions, (3) develop a maintainer for this cms line using the male fertile *cam* cytoplasm from the Polish land race cultivar, Bronowski, and (4) determine the nature of the cytoplasm and the number of nuclear male fertility restorer genes in the Canadian canola cultivar Regent.

Materials and Methods

The nap cms source used in this study was a winter rape (B. napus) line obtained from the National Institute of Agricultural Sciences, Japan (T. Shiga, Nat. Inst. Agric. Sci., Div. Genet., Kannondai, Yatabe, Tsukuba, Ibaraki, 300-21 Japan). The other B. napus cultivars were the Canadian canola cultivar Regent, and the Polish land race cultivar Bronowski.

Evaluation of the Japanese nap cms line was carried out in 1979 and 1980 on the University of Manitoba field plots (Winnipeg , Canada). Plants of the cms line were started in peat pots in late March, grown to the 3 to 4 leaf stage, vernalized for 40 days at 2 to 3° C, hardened for 3 days and then transplanted to the field in early June. Furadan 10G was applied at the time of transplanting for flea beetle control and the transplants were watered. Characteristics associated with the nap male sterile cytoplasm (Shiga, 1976), that is, petal width, stamen length, position of anther with respect to stigma (stamen length divided by pistil length) and a visual male sterility rating (1=male sterile; 2-3=partial male sterile; 4-5=male fertile), were recorded twice weekly throughout flowering on newly opened flowers.

The F2 of a cross between the Japanese cms line and Regent was grown in the field in 1978 and a single male sterile, summer habit plant (MS-1) was found. The MS-1

selection was cloned by rooting stem cuttings in perlite. Evaluation of the MS-1 selection was carried out in the field in 1979. The four male sterile characteristics previously mentioned were recorded twice weekly. The MS-1 selection was self-pollinated using pollen ocassionally produced by the plant in small quantities. Twenty-five progeny from the self (MS-1-1 to MS-1-25) were grown in the greenhouse and self-pollinated. The progenies from self-pollination of MS-1-1 to MS-1-25 were grown in the field in 1980 and evaluated for the aforementioned four male sterile characteristics. Two plants from the progeny of the self of MS-1-5 were selected on the basis of the four characteristics evaluated and overall vigor. These two selections (MS-1-5-1 and MS-1-5-2) were cloned by stem cuttings. The MS-1-5-1 selection was self-pollinated and 25 progeny from the self were grown in the field in 1981. Six plants (MS-1-5-1-1 to MS-1-5-1-6) with the highest degree of male sterility were retained, the remaining plants being rogued out of the plots. These six plants were evaluated for the four male sterile characteristics. These six selected progeny of the self of MS-1-5-1 also were evaluated for effectiveness of pollination control by surrounding the space planted plants with guard rows of Regent, a male fertility restoring cultivar. Using progeny tests of 40 seeds from each of the six selected plants, the proportion of male fertile progeny was taken as an indication of outcrossing frequency.

To develop a maintainer for these nap cms summer rape lines, the progressively improved cms selections were crossed into the male fertile cam cytoplasm of Bronowski using pollen ocassionally produced in small quantities on these selections.

The two B. napus cultivars used in this study, Regent and Bronowski, also were evaluated under field conditions for the four male sterile characteristics from 1979 to 1981 using five plants of each per year.

The cultivar Regent was crossed and the resulting F1 backcrossed to MS-1-5-1 using pollen ocassionally formed in small quantities on this male sterile selection. Progeny of the backcross were evaluated in the greenhouse for male sterility. The nature of the cytoplasm of Regent was determined and an estimate of the number of restorer genes was obtained.

Pearson product-moment correlations among the four male sterile characteristics were calculated.

Results

Evaluation of the Japanese nap cms winter rape (B. napus) line under field conditions in 1979 and 1980 indicated that this line was only partially male sterile under local conditions (Table 1). Petals were narrower, stamens shorter and position of anther with respect to stigma lower on the cms plants than on plants of the male fertile cultivars Regent and Bronowski.

The nap cms summer B. napus selection MS-1 was partially male sterile but had some completely male sterile flowers (Table 1). MS-1 had narrower petals, shorter stamens and a lower position of anther with respect to stigma than the Japanese cms line. The two selections from advanced filial generations of MS-1, namely MS-1-5-1 and MS-1-5-2, were almost completely male sterile under field conditions. The petal width, stamen length and position of anther with respect to stigma of these selections were greater than those for MS-1 but less than those for the Japanese cms line. The six selected progeny from the self of MS-1-5-1 fell into two groups. The first group consisted of MS-1-5-1-2, MS-1-5-1-3 and MS-1-5-1-6. These selections were almost completely male sterile and pollination control was complete (100 % outcrossing). The second group, MS-1-5-1-1, MS-1-5-1-4 and MS-1-5-1-5, was somewhat less than completely male sterile, exhibiting 88 to 95 % outcrossing. MS-1-5-1-2 and MS-1-5-1-3 were the best cms selections when petal width, stamen length and position of anther with respect to stigma were considered. The majority of the anthers on the best cms selections were small cone-shaped structures completely devoid of pollen.

The F1 of the cross between Regent and MS-1-5-1 was male fertile and the progeny of the backcross to MS-1-5-1 segregated 1ms:6pms:192mf. With the assumption of dominance of male fertility restoration at all loci, a 5 complete

restorer gene and 3 partial restorer gene hypothesis (1ms:7pms:248mf) gave the best fit to the observed segregation (X^2 = .12; .90 < p < .95). Thus the cytoplasm of Regent was found to be a nap male sterile cytoplasm and the nuclear genome of Regent apparently contained 5 complete and 3 partial male fertility restorer genes.

The correlations among petal width, stamen length, position of anther with respect to stigma and male sterility rating were positive and highly significant (Table 2).

Discussion

The Japanese nap cms winter rape (B. napus) line was not highly male sterile under local field conditions in 1979 and 1980. Selection within the filial generations of a cross between this line and the Canadian canola cultivar Regent was successful in producing a number of nap cms summer rape lines with a higher degree of male sterility. Some of these lines produced only cross-pollinated seed under field conditions. The floral characteristics of these nap cms summer rape lines were similar to those reported by Shiga (1976) and Bartkowiak-Broda et al. (1979). These included narrow, rugose petals, short stamens, small cone-shaped anthers and a low position of anther with respect to stigma. The low position of anthers with respect to stigma in the male sterile lines would facilitate cross-pollination because it would inhibit self-pollination with the pollen ocassionally produced in small quantities on these plants.

The correlations among petal width, stamen length, position of anther with respect to stigma and male sterility rating were positive and highly significant as observed by Shiga (1976).

Thus it appears that the male sterility in the nap cms material studied can be altered to give a relatively stable system for pollination control under the field conditions in western Canada. The nap male sterile cytoplasm is present in the widely grown Canadian canola cultivar Regent and thus any use of this cytoplasm in hybrids would not involve the risks associated with the use of untested (alien) cytoplasms. However, the major deterant to the use of the nap system is the large number of restorer genes which occur in most B. napus cultivars (Shiga , 1976). For example, the estimated eight genes in Regent that condition male fertility restoration each would have to be changed to their maintainer forms in order to develop a cms form of that cultivar. The male fertile cam cytoplasm of Bronowski could be used to develop the maintainer for such a male sterile but the large number of restorer genes involved would make the development of such a system laborious.

Conclusion

Due to the large number of male fertility restorer genes in most cultivars of *B. napus*, it is difficult to incorporate all the essential agronomic and quality characteristics into the *nap* cms system. However, the system can be stable under field conditions if the restorer alleles at all loci are eliminated. Thus while this system could be used to develop hybrid rape cultivars, other cms systems may prove to be more efficient.

Acknowledgements

The authors gratefully acknowledge financial support from the Canadian Wheat Board, Canola Council of Canada and CSP Foods.

TABLE 1. Field evaluation of the nap cms system in rape (B. napus).

Line	Year	Number of plants	Petal width (mm)	Stamen length (mm)	Anther/stigma#	Male sterility rating^	Outcrossing (%)
Japanese	1979	7	4.8(0.2)$	7.3(0.2)	0.73(0.02)	2-3	-
CMS Line	1980	10	5.0(0.1)	7.9(0.1)	0.88(0.01)	2-3	-
MS-1+	1979	1	3.8(0.1)	6.4(0.4)	0.69(0.04)	2(1)	-
MS-1-5-1	1980	1	4.2(0.4)	6.6(0.4)	0.75(0.01)	1(2)	-
MS-1-5-2	1980	1	4.5(0.2)	6.8(0.6)	0.76(0.04)	1(2)	-
MS-1-5-1-1	1981	1	3.7(0.1)	6.9(0.5)	0.83(0.04)	1-2	88
MS-1-5-1-2	1981	1	3.4(0.1)	5.7(0.4)	0.66(0.03)	1(2)	100
MS-1-5-1-3	1981	1	3.0(0.1)	5.1(0.4)	0.65(0.05)	1(2)	100
MS-1-5-1-4	1981	1	3.6(0.1)	6.8(0.2)	0.81(0.02)	1-2	90
MS-1-5-1-5	1981	1	3.4(0.2)	6.4(0.2)	0.78(0.03)	1-2	95
MS-1-5-1-6	1981	1	3.8(0.1)	6.6(0.4)	0.73(0.03)	1(2)	100
Regent&	1979-81	15	6.6(0.1)	10.1(0.2)	1.32(0.02)	5	-
Bronowski~	1979-81	15	6.0(0.1)	11.6(0.2)	1.25(0.02)	5	-

Position of anther with respect to stigma (stamen length divided by pistil length).
^ Visual male sterility rating:
1 = male sterile,
1(2) = male sterile with ocassional pms flowers,
2(1) = partial male sterile with ocassional ms flowers,
2-3 = partial male sterile,
4-5 = male fertile.
$ Mean(Standard Error of Mean).
+ Male sterile summer rape selection from F2 of (Japanese CMS Line X Regent).
& Canadian canola cultivar with male sterile cytoplasm and nuclear male fertility restorer genes.
~ Polish land race with male fertile cytoplasm and few nuclear male fertility restorer genes.

TABLE 2. Correlations among petal width, stamen length, position of anther with respect to stigma, and male sterility rating (N=12).

	Stamen length	Anther/stigma	Male sterility rating
Petal width	.92**	.91**	.90**
Stamen length		.95**	.94**
Anther/ stigma			.95**

** Highly significant.

WHITE FLOWER COLOR IN RAPE (*BRASSICA NAPUS* L.) ASSOCIATED WITH A RADISH (*RAPHANUS SATIVUS* L.) CHROMOSOME

J. L. SERNYK AND B. R. STEFANSSON

Department of Plant Science,

University of Manitoba,

Winnipeg, Manitoba, Canada R3T 2N2

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Abstract

The inheritance of flower color was investigated in progeny from crosses between a normal, yellow flowered rape strain (*Brassica napus* L.) and a white flowered strain developed at the University of Manitoba by backcrossing the white flower color from *Raphanobrassica* into *B. napus*. The chromosome number of the yellow flowered plants was normal ($2n=38$), while the white flowered plants contained a single or a pair of small radish (*Raphanus sativus* L.) chromosomes each carrying a dominant gene(s) for white flower color. The homozygous white flowered rape strain was in fact an alien addition line ($2n=40$) disomic for a pair of radish chromosomes. The transmission and meiotic behavior of this alien chromosome as a univalent was investigated in the progeny of crosses involving the white flowered F1 hybrid. Cytological observations of meiotic metaphase indicated that the univalent should be transmitted with 24.6% of the gametes. This was in agreement with the observed transmission of 24.3% through the female gametes. Transmission through the pollen ranged from 22.1% to 0.7%, the variation in certation depending on the genetic constitution of the pistil and on the time of pollination. The vigor, fertility and true breeding nature of the white flowered strain suggested that the white flower color factor from the radish had been introgressed into the rape genome.

Cytological observations were necessary to demonstrate that introgression in fact had not taken place and to aid in the interpretation of the genetic ratios observed.

Introduction

Heyn (1979) reported that the white flower color of the white flowered rape strain developed at the University of Manitoba is conditioned by two complementary dominant genes which were introgressed from *Raphanobrassica* into the *Brassica napus* L. genome. He also suggested that one of these two genes for white flower color is linked to one of the male fertility restorer genes for the *ogu* cytoplasmic male sterility (cms) system which is currently being evaluated for use in hybrid rape production (Ogura, 1968; Bartkowiak-Broda *et al.*, 1979; Shiga, 1980).

Kato and Tokumasu (1976) reported that the white flower color in *Raphanus sativus* L. is controlled by a single dominant gene. Because the *Raphanobrassica* used as the source of the white flower color was developed from a cross between a white flowered *R. sativus* ($2n=18$) and a yellow flowered *Brassica oleracea* L. ($2n=18$), only a single dominant gene would be expected to be involved in the control of the white flower color in the derived rape strain.

Genetic and cytological investigations were undertaken to determine the inheritance of this trait.

Materials and Methods

The true breeding white flowered strain of *B. napus* used in this study was developed at the University of Manitoba by backcrossing the white flower color from *Raphanobrassica* (*R. sativus* x *B. oleracea*), obtained from the University of Minnesota (R.E. Comstock, Dept. Genet. Cell Biol., Coll. Biol. Sci., Univ. Minn., St. Paul, MN 55108), into *B. napus*.

True breeding white and true breeding yellow strains of rape were hybridized to produce F1, F2 and BC generations. All crosses were made reciprocally. Selfing was accomplished by bagging racemes and tapping the bags daily to ensure pollination. Crossing was done by emasculating the immature flower buds of the female parent, and then immediately pollinating the stigmas with freshly dehiscent pollen from the male parent (bud pollination). To determine the effect of delayed pollination, extra sets of crosses were made by emasculating the immature flower buds of the female parent, bagging the inflorescences and then pollinating the stigmas with freshly dehiscent pollen from the male parent when the pistils were the size of the pistils of newly opened flowers (about 1 cm long). Progenies of each of these generations were grown and flower color counts were made.

Pollen mother cells (PMCs) from the parents, F1, and selected F2 plants were examined using light microscopy. For this purpose, flower buds 1.4 to 1.6 mm long were

collected and fixed in Carnoy's fixative (6 parts 95% ethanol: 3 parts chloroform: 1 part glacial acetic acid). For cytological studies, anthers were removed and squashed on slides using a 1% aceto-carmine solution. Chromosome counts and drawings of meiotic chromosomes were made using these preparations.

Results

Genetic Data

The white and yellow flowered strains were true breeding prior to and throughout the study. The white flowered strain was vigorous and showed no evidence of reduced female or male fertility.

All 59 F1 plants from the White X Yellow and Yellow X White crosses produced white flowers, indicating dominance of white over yellow flower color. All 347 plants of the backcrosses of the F1 to the white flowered parent were white as would be expected.

However, as the F2 data and chi-square tests in Table 1 indicate, the F2 segregation did not fit either the single dominant gene (3W:1Y) or the two complementary dominant gene (9W:7Y) hypotheses. Similarly, the backcross data and chi-square tests in Table 1 indicate that there were significant differences between reciprocal backcrosses: the data from the backcross with the F1 as the female parent fit the two complementary dominant gene hypothesis (1W:3Y), but the reciprocal backcross data fit neither the single

dominant gene (1W:1Y) nor the two complementary dominant gene (1W:3Y) hypothesis. There was also a significant difference in the observed ratios between bud (1W:5Y) and delayed (1W:134Y) pollination in the backcrosses with the F1 as the male parent. The heterogeneity chi-squares were not significant in all cases and consequently the data were pooled; only the chi-square tests for the pooled data were reported.

The frequency of transmission for the white flower color factor through the female and the male gametes was determined from the progeny of the appropriate crosses (Table 1). Transmission through the female gametes was obtained directly from the frequency of white flowered plants in the progeny of the crosses of heterozygous white flowered females (F1) and yellow flowered pollen parents. The reciprocals of these crosses provided data on the transmission through the male gametes. In addition, transmission of the white flower color factor through the pollen into a heterozygous female was calculated from the F2 data using the assumption that transmission through the female gamete should be independent of the pollen source. Transmission of the white flower color factor through the female gametes was 24.3% [(124+86)/(124+86+418+237)], while transmission through the male gametes varied from 22.1% [1-(1025/(712+1025))/(1-.243)] when the female parent carried the white flower color factor, to 16.7% [50/(50+250)] for bud pollination onto a yellow flowered

female, to 0.7% [1/(1+134)] for delayed pollination onto a yellow flowered female (Table 2).

Yellow sectors in the white petals were observed ocassionally on plants heterozygous for the white flower color factor.

These data suggest that some chromosomal abnormality may be involved in the inheritance of this white flower color factor.

Cytological Observations

Examination of PMCs of the white and yellow flowered strains and the white flowered F1 indicated that the white strain had 20 II chromosomes; the yellow strain, 19 II; and the white F1, 19 II + 1 I (Figure 1). Furthermore, examination of PMCs of 30 white and yellow flowered F2 progeny indicated that all yellow flowered progeny had 19 II, while the white flowered progeny had either 19 II + 1 I or 20 II (Table 3). At least 25 PMCs per plant were examined to obtain these results.

These cytological observations indicated that white flower color was invariably associated with the presence of a small, alien Raphanus chromosome. The transmission frequencies obtained from the genetic data thus reflected the transmission of the univalent alien chromosome through the female and male gametes. The true breeding nature of the homozygous white strain suggested that the transmission

of the alien chromosome was normal when present in the bivalent condition.

Cytological observations of the univalent Raphanus chromosome during meiotic Metaphase I in the white flowered F1 indicated that the univalent was not aligned on the plate in 61 out of 120 PMCs (50.8%) observed. Assuming that nonaligned univalents would be lost from the gametes produced, and that in those PMCs with aligned univalents only one-half of the gametes produced would receive the extra chromosome, it was possible to estimate the rate of incorporation of the univalent into gametes as 24.6% [(100-50.8)/2].

Discussion

Inheritance of the white flower color factor in the white flowered B. napus strain developed at the University of Manitoba appears to be controlled by a dominant (inhibitor) gene(s) located on a small, alien chomosome derived from R. sativus. This white flowered strain is a disomic alien addition line ($2n=40$) in contrast to the normal, yellow flowered B. napus ($2n=38$).

Kato and Tokumasu (1976) reported that a single dominant gene controlled white flower color in R. sativus. Thus the white flowered B. napus strain in the present study must contain the Raphanus chromosome carrying the gene for white flower color. The suggestion by Heyn (1979) of a two

complementary dominant gene model probably resulted from examination only of backcrosses of the white F1 (female) to the yellow parent (male) which fits the expected 1W:3Y ratio (Table 1).

The presence of the additional chromosome as a univalent in the white F1 permitted a study of the incorporation and transmission of the alien univalent in both the female and male gametes. The cytological observations indicated a rate of incorporation of the univalent chromosome into the gametes of 24.6% which is in agreement with the 24.3% transmission rate through the female gametes. This result is expected because transmission of the univalent through the female gametes should depend solely on its behavior during meiosis. Its transmission through the male gametes, however, depends not only on its meiotic behavior, but also on certation effects which may vary depending on the genotype of the pollen and pistil and also on the time of pollination. Observations of meiosis indicated that the univalent should be incorporated into about 24.6% of the male gametes, with any reduction below this level being due to certation. The level of certation observed is minimal when the pistil also contains the white flower color factor (22.1% transmission), is moderate when the pistil lacks the white flower color factor and is bud pollinated (16.7% transmission), and is extreme when the pistil lacks the white flower color factor and pollination is delayed (0.7% transmission).

These results are similar to those reported by Sears (1954) for wheat monosomics. He observed 25% transmission of the monosomic chromosome through the female gametes and from 0 to 10% transmission through the male gametes.

The evidence presented demonstrates that the white flowered rape strain is a chromosome addition line and that the white flower color gene has not been introgressed into the rape genome as suggested by Heyn (1979). Both genetic and cytological data were required to distinguish between these possibilities.

The possibilities of developing hybrid rape cultivars may depend on the development of cms systems using alien cytoplasms such as the ogu and mur cytoplasms, and the transfer of male fertility restorer genes from alien species (Heyn , 1979; Bartkowiak-Broda et al., 1979; Shiga , 1980). Introgression of these fertility restorer genes into the rape genome is desirable because a chromosome addition line such as the one described in this report probably would carry undesirable genetic information on the alien chromosome. The evidence from the present study indicates that introgression of such alien genes might not be easy to accomplish and that both genetic and cytological investigations are required to determine if introgression has in fact taken place.

Acknowledgments

Financial support from the Canadian Wheat Board and the Department of Plant Science, University of Manitoba is gratefully acknowledged.

TABLE 1. Observed segregation and chi-square tests for F2 and BC data from crosses of *B. napus* involving white and yellow flower color.

Cross and time of pollination	Number of sets	Observed segregation White	Observed segregation Yellow	χ^2 F2 - 3W:1Y BC - 1W:1Y	P	χ^2 F2 - 9W:7Y BC - 1W:3Y	P
F2	12	712	1025	1,071.5	<0.005	164.4	<0.005
BC : (WxY)xY (bud)	6	124	418	159.5	<0.005	1.3	0.25-0.50
BC : (WxY)xY (delayed)	4	86	237	70.6	<0.005	0.5	0.25-0.50
BC : Yx(WxY) (bud)	4	50	250	133.3	<0.005	11.1	<0.005
BC : Yx(WxY) (delayed)	4	1	134	131.0	<0.005	42.4	<0.005

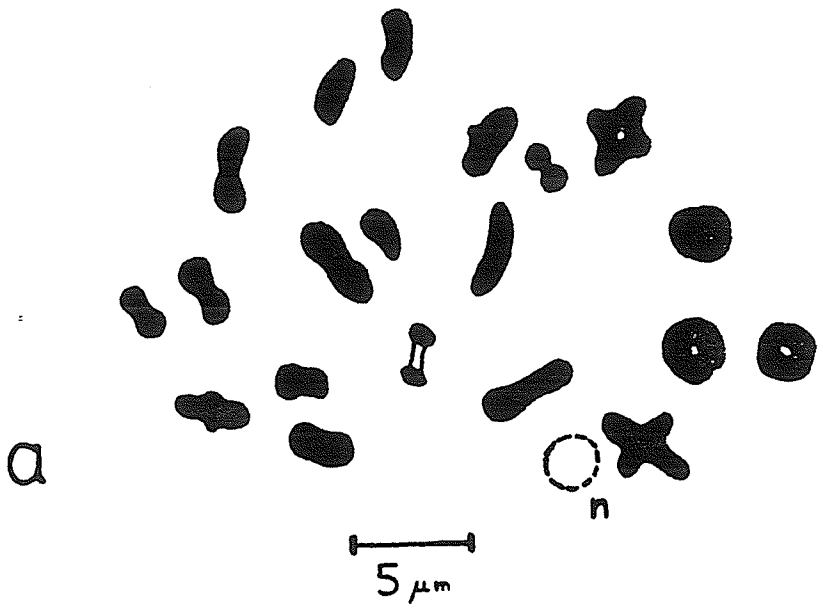
TABLE 2. Transmission of the white flower color factor through female and male gametes in different crosses.

Gamete	Cross and time of pollination	Transmission (% of gametes)
Female	F1 x Y (bud and delayed)	24.3
Male	F1 x F1 (bagging)	22.1
Male	Y x F1 (bud)	16.7
Male	Y x F1 (delayed)	0.7

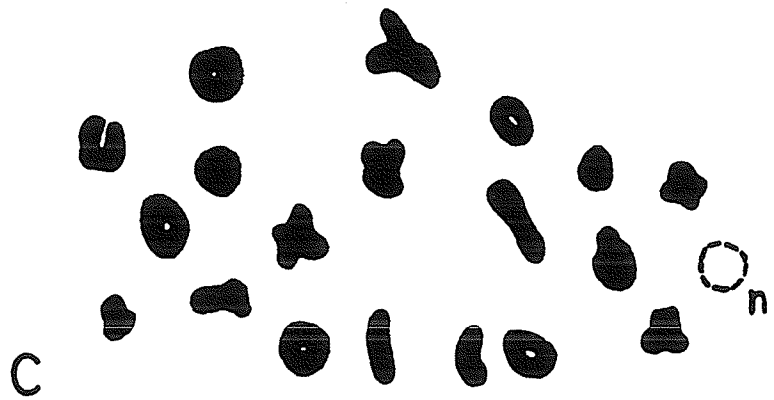
TABLE 3. Chromosome number of a sample of white and yellow flowered F2 progeny.

Flower color	Number of progeny with		
	19 II	19 II + 1 I	20 II
White	0	17	2
Yellow	11	0	0

Figure 1. PMCs of B. napus: (a) white flowered strain - 20 II, (b) white flowered F1 - 19 II + 1 I (univalent is indicated with arrow), and (c) yellow flowered strain - 19 II. Nucleoli are also indicated (n).


a
n
5 μm


b


c
n

GENERAL DISCUSSION

The two main requirements for commercial production of F1 hybrids of rape (B. napus) were investigated in three years of field trials (1979 to 1981). These two requirements are: (1) heterosis or hybrid vigor and (2) a pollination control system.

Heterosis

The first requirement, that of sufficient heterosis or hybrid vigor to justify the added costs involved in hybrid seed production, was investigated in replicated yield trials in 1980 and 1981 using hand-crossed intervarietal hybrids. The degree of heterosis consistently observed in F1 hybrids during these two years of testing suggested that it may be possible to develop hybrid cultivars of B. napus with the potential of producing seed yields 40 % higher than those of the conventional canola cultivar Regent. These findings were in agreement with the results of other researchers who have indicated that heterosis for seed yield of 40 to 60% is available in F1 hybrids of B. napus (Schuster and Michael, 1976; Shiga, 1976; Morice, 1978; Buson, 1980; Guan, 1980).

These high yielding hybrids were very vigorous and thus emerged and developed a crop canopy more rapidly than

Regent. Early flowering, early maturity and tall stature were partially dominant in these hybrids. In spite of the yield advantage, resistance to lodging for the hybrids was equal to or better than that for Regent. Thousand seed weight, seed oil content and seed protein content for the hybrids were comparable to those for Regent.

The requirement for canola quality in Canada and the known inheritance of the characteristics involved dictate that both parents must be low in the erucic acid content of the oil and in the glucosinolate content of the seed so that the seed from the F1 hybrid will be of acceptable quality (Jonsson , 1977; Kondra and Stefansson , 1970). Thus the two double-low (low erucic acid, low glucosinolate) hybrid combinations, Marnoo X Regent and Karat X Regent, are of particular interest. They exhibit, respectively, 38 % and 43 % heterosis for seed yield over Regent, are of canola quality, and are only 1 day later maturing than Regent. The Gullivar X Regent hybrid also showed significant heterosis (40 to 43 % over Regent), but was not of canola quality due to the high glucosinolate genotype of the Gullivar parent. The level of heterosis for seed yield (approximately 40 %) obtained for certain F1 hybrids of rape (B. napus) thus appears to be sufficient to justify the added costs involved in hybrid seed production.

The vigor of these high yielding hybrids of B. napus also was evident in total dry matter production which was 25 to

29 % greater than that for Regent. The heterosis observed for apparent harvest index indicated that these hybrids also were able to convert a greater proportion of this increased total dry matter yield to seed production.

Positive correlations among seed yield, total dry matter yield, and harvest index are typical of the responses of a crop to water when grown under suboptimal moisture conditions (Donald and Hamblin , 1976). Thus the highly significant, positive correlations observed among seed yield, total dry matter yield, and apparent harvest index suggested that summer rape (B. napus) in western Canada responds significantly to water and that the hybrids were superior to Regent in their ability to utilize soil water resources. Such a suggestion also was supported by the work of Guan (1980) which indicated that hybrids of B. napus were superior to parental lines in exuding water rate, a measure of water translocation.

The observed increases in seed yield, total dry matter yield, and apparent harvest index of the better hybrids occurred without any significant lengthening of the reproductive growth stage. This suggests that the vigor observed in the hybrids prior to flowering continued throughout the reproductive growth period. Although root development was not studied, the vigor in top growth and the superior ability of the hybrids to exploit soil water resources suggested that the hybrids also possessed a vigorous root system.

Pollination Control System

The second requirement for commercial F1 hybrid production, that of a pollination control system, was investigated in the field for 3 years (1979 to 1981). The studies involved the *nap* cytoplasmic male sterility (cms) system and had four main objectives: (1) evaluation of the *nap* cms system under local field conditions, (2) development of a summer habit *nap* cms line which would be stable under local field conditions, (3) development of a maintainer for this cms line using the male fertile *cam* cytoplasm, and (4) determination of the nature of the cytoplasm and the number of male fertility restorer genes in the Canadian canola cultivar Regent.

The Japanese *nap* cms winter rape (*B. napus*) line obtained from Shiga was not highly male sterile under local field conditions in 1979 and 1980. Selection within the filial generations of a cross between the Japanese cms line and the Canadian canola cultivar Regent was successful in producing a number of *nap* cms summer rape lines with a higher degree of male sterility. Some of these lines produced only cross-pollinated seed under field conditions. A maintainer for these male sterile lines was developed by backcrossing these selections into the male fertile *cam* cytoplasm of Bronowski.

The floral characteristics of these *nap* cms summer rape lines were similar to those reported by Shiga (1976) and

Bartkowiak-Broda *et al*. (1979). These included narrow rugose petals, short stamens, small cone-shaped anthers and a low position of anther with respect to stigma. The low position of anther with respect to stigma in the male sterile lines facilitated cross-pollination because it would act to prevent self-pollination with the pollen ocassionally produced in small quantities on these plants. The correlations among petal width, stamen length, relative position of anther with respect to stigma and male sterility rating were positive and highly significant as observed by Shiga (1976).

Thus it appeared that the male sterility in the *nap* cms material studied could be modified to give a relatively stable system for pollination control under the field conditions in western Canada. The *nap* male sterile cytoplasm is present in the widely grown Canadian canola cultivar Regent and thus any use of this cytoplasm in hybrids would not involve the risks associated with the use of untested (alien) cytoplasms. However, the major dificulty with the *nap* system is the large number of restorer genes which occur in most *B. napus* cultivars (Shiga , 1976). For example, the Canadian canola cultivar Regent, which has potential for use in hybrids, was estimated to have 5 complete and 3 partial male fertility restorer genes. Thus to develop a cms form of the cultivar Regent for use in hybrid production, these 8 restorer genes would have to be

changed to their maintainer forms while retaining the yield, quality, agronomic characteristics and combining ability of Regent. The male fertile cam cytoplasm of Bronowski could be used to develop the maintainer for such a male sterile but the large number of restorer genes involved would make the development of such a system laborious.

Thus due to the large number of male fertility restorer genes in most cultivars of B. napus, it would be difficult to incorporate all the essential agronomic and quality characteristics into the nap cms system. However the system can be stable under field conditions if the restorer alleles at all loci are eliminated. Thus while this system could be used to develop hybrid rape cultivars, other cms systems may prove to be more efficient.

The other two cms systems currently being evaluated for possible use in F1 hybrid production of rape are the ogu and the mur cms systems. Both of these are alien cms systems originating from R. sativus and D. muralis respectively, and will require the incorporation of nuclear male fertility restorer genes from the donor species (Shiga, 1980). In view of this requirement, the inheritance of an alien white flower color factor(s) introduced into B. napus from R. sativus via Raphanobrassica was investigated. Heyn (1979) had suggested that this trait involved two genes which had been introgressed into the B. napus genome. He also suggested that one of these two genes was linked with one of

the two genes for male fertility restoration in the ogu cms system.

The evidence obtained demonstrates that the inheritance of the white flower color factor in the white flowered B. napus strain developed at the University of Manitoba is controlled by a dominant (inhibitor) gene(s) located on a small, alien chomosome derived from R. sativus. This white flowered strain was found to be a disomic alien addition line (2n=40) in contrast to the normal, yellow flowered B. napus (2n=38).

Kato and Tokumasu (1976) reported that a single dominant gene controlled white flower color in R. sativus. Thus the white flowered B. napus strain used in this study must contain the Raphanus chromosome carrying the gene for white flower color. The suggestion by Heyn (1979) of a two complementary dominant gene model probably resulted from evaluating the backcrosses of the white F1 (female) to the yellow parent (male) which fit the expected 1W:3Y ratio without adequate consideration of other generations.

The presence of the additional chromosome as a univalent in the white F1 permitted a study of the incorporation and transmission of the alien univalent in both the female and male gametes. The cytological observations indicated a rate of incorporation of the univalent chromosome into the gametes of 24.6% which is in agreement with the 24.3% transmission rate through the female gametes. This result

was expected since transmission of the univalent through the female gametes should depend solely on its behavior during meiosis. Its transmission through the male gametes, however, depends not only on its meiotic behavior, but also on certation effects which may vary depending on the genotype of the pollen and pistil and also on the time of pollination. Observations of meiosis indicated that the univalent should be incorporated into about 24.6% of the male gametes, with any reduction below this level being due to certation. The level of certation observed is minimal when the pistil also contains the white flower color factor (22.1% transmission), is moderate when the pistil lacks the white flower color factor and is bud pollinated (16.7% transmission), and is extreme when the pistil lacks the white flower color factor and pollination is delayed (0.7% transmission).

These results are similar to those reported by Sears (1954) for wheat monosomics. He observed 25% transmission of the monosomic chromosome through the female gametes and from 0 to 10% transmission through the male gametes.

Thus the evidence indicates that the white flowered rape strain is a chromosome addition line and that the white flower color gene has not been introgressed into the rape genome as suggested by Heyn (1979). Both genetic and cytological data were required to distinguish between these possibilities.

In the case of alien cms systems, introgression of the alien fertility restorer genes into the rape genome is desirable since a chromosome addition line such as the one described in this thesis probably would carry undesirable genetic information on the alien chromosome. The evidence obtained suggests that introgression of such alien genes might not be easy to accomplish and that both genetic and cytological investigations are required to determine if introgression has in fact taken place.

SUMMARY AND CONCLUSIONS

The investigations of heterosis in summer rape (B. napus) demonstrated that F1 hybrids can be produced with acceptable agronomic characteristics, canola quality and 40% yield advantage over the conventional canola cultivar Regent. Such hybrids are very vigorous due to increased ability to exploit their environment (water, nutrients and solar radiation). They also demonstrate the ability to yield a greater proportion of their dry matter production as seed.

The investigation of the nap cytoplasmic male sterility (cms) system indicated that although male sterility in this system can be stabilized under field conditions, the large number of male fertility restorer genes in most B. napus cultivars (e.g. 8 restorer genes in Regent) would make the system very difficult to use. The mur cms system seems to have the best potential for use in hybrid rape production as this system has complete sterility and only a single restorer gene.

The investigation of the "introgressed" white flower color in B. napus indicated that this trait had not been introgressed into the rape genome but instead was present on an additional alien chromosome. The inheritance of this alien chromosome was normal from the disomic condition but

from the monosomic condition was similar to that observed for monosomics in other crops. These results suggest that future introgression of alien traits such as nuclear male-fertility restorers may be difficult and that both genetic and cytological studies will be necessary to determine if introgression has in fact taken place.

These results suggest that there is potential for F1 hybrid rape (*B. napus*) production and that the development of a reliable and easy to use pollination control system is the main limiting factor.

SUGGESTIONS FOR FURTHER INVESTIGATIONS

While the investigations of this thesis research have considered some of the factors essential in the production of hybrid rape (*B. napus*) cultivars, much work remains to be done. The following could be fruitful areas for investigation:

1. Nature of heterosis:
    a) The effects of heterosis on seed quality (fatty acid composition, glucosinolate levels, etc.).
    b) The levels of heterosis in three-way cross hybrids.
    c) The development of early prediction techniques for levels of heterosis (seedling tests).
    d) The effect of the different cms systems on the level of heterosis.
    e) The genetics of heterosis (gene action, general and specific combining abilities).
    f) The effects of inbreeding on heterosis in F2 and subsequent generations.
    g) The effects of mixtures of different proportions of hybrid and parental seed on the level of heterosis.

h) The physiology of heterosis (growth analysis, photosynthesis, water use efficiency).

i) The effects of fertilizer and seeding rates on heterosis.

2. Characteristics of cytoplasmic male sterility (cms) systems:

a) The investigation of the meiotic behavior of alien restorer genes in these systems.

b) The possible association of disease and/or insect susceptibility with these male sterile cytoplasms.

c) The attraction of insect pollinators in these cms systems (absense of nectaries, nectar production, sugar concentration, importance of pollen).

d) The determination of the cytoplasmic organelle associated with male sterility in these systems.

e) The search for other cms systems using related species.

3. Development of hybrid cultivars:

a) The development of the ogu and mur cms systems.

b) The development of tester populations to facilitate the testing of potential parents for hybrids.

c) The development of inbred lines for use in hybrids.

d) The investigation of all aspects of field scale hybrid seed production (single-cross hybrids,

three-way cross hybrids, the ratio of female to male rows, insect pollinators).

Investigations into the potential of hybrid cultivars in rape (*B. napus*) have been initiated but much more basic knowledge must be obtained before hybrid cultivars can be produced efficiently.

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APPENDIX

TABLE 1. Temperature and precipitation data for the 1980 and 1981 hybrid rape (*B. napus*) yield trials.

Time period	1980		1981	
	Average temperature (Celcius)	Precipitation (mm)	Average temperature (Celcius)	Precipitation (mm)
May 21-31	22.1	13	12.9	60
Jun 1-30	16.8	155#	16.0	84
Jul 1-31	20.4	19	20.3	49
Aug 1-31	16.9	115	20.1	107
Sep 1-6	16.4	24	13.8	36
Season	18.4	326	18.0	336

Includes 102 mm of irrigation water applied June 19-22.

TABLE 2. Soil test results for the 1980 and 1981 hybrid rape (*B. napus*) yield trials.

Depth (cm)	Texture	Carbonate content	pH	Salinity (mmhos/cm)	Nitrate nitrogen (kg/ha)	Available phosphorous (kg/ha)	Available potassium (kg/ha)	Sulphate sulphur (kg/ha)
0-15	clay	low	7.7	0.8	45.7	26.3	913	36+
15-60	clay	high		0.8	145.3			126+

Analyses done by the Manitoba Provincial Soil Testing Laboratory, Winnipeg, Canada.

TABLE 3. ANOVA# and contrasts for Days from seeding to emergence.

Seeding date	Source	D F	M S	F-value
May 21, 1980	Block	2	4.40	1.11
	Line/Hybrid	8	2.67	0.67
	C1^	1	4.00	1.01
	C2	1	1.78	0.45
	C3	1	0.11	0.03
	C4	1	1.00	0.25
	Error	37	3.96	
	Total	47		
May 20, 1981	Block	2	4.23	9.84**
	Line/Hybrid	4	2.76	6.42**
	C1	1	10.03	23.33**
	C5	1	2.25	5.23*
	Error	23	0.43	
	Total	29		
June 8, 1981	Block	2	0.03	0.50
	Line/Hybrid	4	3.63	60.50**
	C6	1	0.00	0.00
	C2	1	0.00	0.00
	Error	23	0.06	
	Total	29		

Analysis of variance.

^ Contrasts:
C1 = Regent vs Regent x Gullivar
C2 = Regent vs Regent x Schuster
C3 = Regent vs Regent x Kosa
C4 = Regent vs Regent x Bronowski
C5 = Regent vs Regent x Marnoo
C6 = Regent vs Regent x Karat

* Significant
** Highly significant

TABLE 4. ANOVA and contrasts for Days from emergence to flowering.

Seeding date	Source	D F	M S	F-value
May 21, 1980	Block	2	5.15	3.37*
	Line/Hybrid	8	63.70	41.62**
	C1#	1	6.25	4.08
	C2	1	51.36	33.57**
	C3	1	6.25	4.08
	C4	1	51.36	33.57**
	Error	37	1.53	
	Total	47		
May 20, 1981	Block	2	17.73	50.66**
	Line/Hybrid	4	26.65	76.14**
	C1	1	8.03	22.94**
	C5	1	8.03	22.94**
	Error	23	0.35	
	Total	29		
June 8, 1981	Block	2	3.23	2.86
	Line/Hybrid	4	138.64	122.69**
	C6	1	0.25	0.22
	C2	1	23.36	20.67**
	Error	23	1.13	
	Total	29		

# Contrasts:
C1 = Regent vs Regent x Gullivar
C2 = Regent vs Regent x Schuster
C3 = Regent vs Regent x Kosa
C4 = Regent vs Regent x Bronowski
C5 = Regent vs Regent x Marnoo
C6 = Regent vs Regent x Karat

* Significant
** Highly significant

TABLE 5. ANOVA and contrasts for Days from emergence to maturity.

Seeding date	Source	D F	M S	F-value
May 21, 1980	Block	2	4.02	1.23
	Line/Hybrid	8	30.83	9.40**
	C1#	1	26.69	8.14**
	C2	1	30.25	9.22**
	C3	1	1.36	0.41
	C4	1	25.25	7.70**
	Error	37	3.28	
	Total	47		
May 20, 1981	Block	2	9.70	14.92**
	Line/Hybrid	4	15.97	24.57**
	C1	1	14.69	22.60**
	C5	1	4.69	7.22*
	Error	23	0.65	
	Total	29		
June 8, 1981	Block	2	4.23	5.10*
	Line/Hybrid	4	156.09	188.06**
	C6	1	2.78	3.35
	C2	1	28.44	34.27**
	Error	23	0.83	
	Total	29		

# Contrasts:
C1 = Regent vs Regent x Gullivar
C2 = Regent vs Regent x Schuster
C3 = Regent vs Regent x Kosa
C4 = Regent vs Regent x Bronowski
C5 = Regent vs Regent x Marnoo
C6 = Regent vs Regent x Karat

* Significant
** Highly significant

TABLE 6. ANOVA and contrasts for Height at maturity (cm).

Seeding date	Source	D F	M S	F-value
May 21, 1980	Block	2	84.02	3.44*
	Line/Hybrid	8	390.27	15.97**
	C1#	1	860.44	35.21**
	C2	1	1089.00	44.56**
	C3	1	693.44	28.37**
	C4	1	1133.44	46.38**
	Error	37	24.44	
	Total	47		
May 20, 1981	Block	2	153.90	8.16**
	Line/Hybrid	4	127.83	6.78**
	C1	1	300.44	15.93**
	C5	1	169.00	8.96**
	Error	23	18.86	
	Total	29		
June 8, 1981	Block	2	81.90	2.45
	Line/Hybrid	4	289.37	8.67**
	C6	1	513.78	15.40**
	C2	1	513.78	15.40**
	Error	23	33.37	
	Total	29		

# Contrasts:
C1 = Regent vs Regent x Gullivar
C2 = Regent vs Regent x Schuster
C3 = Regent vs Regent x Kosa
C4 = Regent vs Regent x Bronowski
C5 = Regent vs Regent x Marnoo
C6 = Regent vs Regent x Karat

* Significant
** Highly significant

TABLE 7. ANOVA and contrasts for Lodging at maturity (1-5).

Seeding date	Source	D F	M S	F-value
May 21, 1980	Block	2	0.77	3.85*
	Line/Hybrid	8	1.14	5.70**
	C1#	1	0.44	2.20
	C2	1	0.44	2.20
	C3	1	0.44	2.20
	C4	1	5.44	27.20**
	Error	37	0.20	
	Total	47		
May 20, 1981	Block	2	0.00	-
	Line/Hybrid	4	0.00	-
	C1	1	0.00	-
	C5	1	0.00	-
	Error	23	0.00	
	Total	29		
June 8, 1981	Block	2	1.23	3.32
	Line/Hybrid	4	2.97	8.03**
	C6	1	0.69	1.86
	C2	1	0.03	0.08
	Error	23	0.37	
	Total	29		

# Contrasts:
C1 = Regent vs Regent x Gullivar
C2 = Regent vs Regent x Schuster
C3 = Regent vs Regent x Kosa
C4 = Regent vs Regent x Bronowski
C5 = Regent vs Regent x Marnoo
C6 = Regent vs Regent x Karat

* Significant
** Highly significant

TABLE 8. ANOVA and contrasts for Seed yield (kg/ha).

Seeding date	Source	D F	M S#	F-value
May 21, 1980	Block	2	17.97	1.40
	Line/Hybrid	8	81.51	6.33**
	C1^	1	305.39	23.75**
	C2	1	42.77	3.33
	C3	1	9.77	0.76
	C4	1	0.59	0.05
	Error	37	12.86	
	Total	47		
May 20, 1981	Block	2	4.79	0.65
	Line/Hybrid	4	96.27	13.15**
	C1	1	173.48	23.70**
	C5	1	133.14	18.19**
	Error	23	7.32	
	Total	29		
June 8, 1981	Block	2	10.84	1.77
	Line/Hybrid	4	32.88	5.36**
	C6	1	85.91	14.01**
	C2	1	15.27	2.49
	Error	23	6.13	
	Total	29		

MS values are divided by 10,000.

^ Contrasts:
C1 = Regent vs Regent x Gullivar
C2 = Regent vs Regent x Schuster
C3 = Regent vs Regent x Kosa
C4 = Regent vs Regent x Bronowski
C5 = Regent vs Regent x Marnoo
C6 = Regent vs Regent x Karat

* Significant
** Highly significant

TABLE 9. ANOVA and contrasts for Thousand seed weight (gm).

Seeding date	Source	D F	M S	F-value
May 21, 1980	Block	2	0.006	0.17
	Line/Hybrid	8	0.768	21.33**
	C1#	1	0.010	0.27
	C2	1	0.871	24.19**
	C3	1	0.010	0.27
	C4	1	1.000	27.78**
	Error	37	0.036	
	Total	47		
May 20, 1981	Block	2	0.012	1.00
	Line/Hybrid	4	0.053	4.42**
	C1	1	0.031	2.58
	C5	1	0.029	2.42
	Error	23	0.012	
	Total	29		
June 8, 1981	Block	2	0.242	11.52**
	Line/Hybrid	4	0.278	13.24**
	C6	1	0.499	23.76**
	C2	1	0.518	24.67**
	Error	23	0.021	
	Total	29		

# Contrasts:
C1 = Regent vs Regent x Gullivar
C2 = Regent vs Regent x Schuster
C3 = Regent vs Regent x Kosa
C4 = Regent vs Regent x Bronowski
C5 = Regent vs Regent x Marnoo
C6 = Regent vs Regent x Karat

* Significant
** Highly significant

TABLE 10. ANOVA and contrasts for Oil content of seed (%).

Seeding date	Source	D F	M S	F-value
May 21, 1980	Block	2	3.13	5.40**
	Line/Hybrid	8	7.13	12.29**
	C1#	1	0.07	0.12
	C2	1	9.61	16.57**
	C3	1	2.25	3.88
	C4	1	8.60	14.83**
	Error	37	0.58	
	Total	47		
May 20, 1981	Block	2	2.13	4.63*
	Line/Hybrid	4	24.51	53.28**
	C1	1	2.78	6.04*
	C5	1	2.67	5.80*
	Error	23	0.46	
	Total	29		
June 8, 1981	Block	2	3.10	6.89**
	Line/Hybrid	4	1.91	4.24*
	C6	1	0.20	0.44
	C2	1	0.10	0.22
	Error	23	0.45	
	Total	29		

# Contrasts:
C1 = Regent vs Regent x Gullivar
C2 = Regent vs Regent x Schuster
C3 = Regent vs Regent x Kosa
C4 = Regent vs Regent x Bronowski
C5 = Regent vs Regent x Marnoo
C6 = Regent vs Regent x Karat

* Significant
** Highly significant

TABLE 11. ANOVA and contrasts for Protein content of seed (%).

Seeding date	Source	D F	M S	F-value
May 21, 1980	Block	2	2.21	8.19**
	Line/Hybrid	8	4.74	17.56**
	C1#	1	1.00	3.70
	C2	1	26.35	97.59**
	C3	1	7.11	26.33**
	C4	1	5.76	21.33**
	Error	37	0.27	
	Total	47		
May 20, 1981	Block	2	5.39	14.57**
	Line/Hybrid	4	15.21	41.11**
	C1	1	2.56	6.92*
	C5	1	9.40	25.41**
	Error	23	0.37	
	Total	29		
June 8, 1981	Block	2	0.74	1.21
	Line/Hybrid	4	13.10	21.48**
	C6	1	0.44	0.72
	C2	1	13.94	22.85**
	Error	23	0.61	
	Total	29		

# Contrasts:
C1 = Regent vs Regent x Gullivar
C2 = Regent vs Regent x Schuster
C3 = Regent vs Regent x Kosa
C4 = Regent vs Regent x Bronowski
C5 = Regent vs Regent x Marnoo
C6 = Regent vs Regent x Karat

* Significant
** Highly significant

TABLE 12. ANOVA and contrasts for Total dry matter yield (kg/ha).

Seeding date	Source	D F	M S#	F-value
May 21, 1980	Block	2	555.31	5.91**
	Line/Hybrid	8	304.25	3.24**
	C1^	1	2046.61	21.78**
	C2	1	676.05	7.19*
	C3	1	156.87	1.67
	C4	1	246.44	2.62
	Error	37	93.98	
	Total	47		
May 20, 1981	Block	2	5.97	0.14
	Line/Hybrid	4	420.76	10.12**
	C1	1	1178.20	28.34**
	C5	1	961.03	23.11**
	Error	23	41.58	
	Total	29		
June 8, 1981	Block	2	256.76	3.62*
	Line/Hybrid	4	300.42	4.24*
	C6	1	839.27	11.84**
	C2	1	142.62	2.01
	Error	23	70.88	
	Total	29		

MS values are divided by 10,000.

^ Contrasts:
C1 = Regent vs Regent x Gullivar
C2 = Regent vs Regent x Schuster
C3 = Regent vs Regent x Kosa
C4 = Regent vs Regent x Bronowski
C5 = Regent vs Regent x Marnoo
C6 = Regent vs Regent x Karat

* Significant
** Highly significant

TABLE 13. ANOVA and contrasts for Apparent harvest index (%).

Seeding date	Source	D F	M S	F-value
May 21, 1980	Block	2	6.61	1.37
	Line/Hybrid	8	57.64	11.96**
	C1#	1	25.90	5.37*
	C2	1	0.25	0.05
	C3	1	0.08	0.02
	C4	1	34.07	7.07*
	Error	37	4.82	
	Total	47		
May 20, 1981	Block	2	16.18	3.29
	Line/Hybrid	4	68.04	13.83**
	C1	1	37.01	7.52*
	C5	1	23.02	4.68*
	Error	23	4.92	
	Total	29		
June 8, 1981	Block	2	1.49	0.13
	Line/Hybrid	4	71.81	6.34**
	C6	1	32.80	2.90
	C2	1	9.40	0.83
	Error	23	11.32	
	Total	29		

# Contrasts:
C1 = Regent vs Regent x Gullivar
C2 = Regent vs Regent x Schuster
C3 = Regent vs Regent x Kosa
C4 = Regent vs Regent x Bronowski
C5 = Regent vs Regent x Marnoo
C6 = Regent vs Regent x Karat

* Significant
** Highly significant

TABLE 14. ANOVA and contrasts for Reproductive growth period (%).

Seeding date	Source	D F	M S	F-value
May 21, 1980	Block	2	7.02	4.68*
	Line/Hybrid	8	39.17	26.11**
	C1#	1	0.27	0.18
	C2	1	26.58	17.72**
	C3	1	4.71	3.14
	C4	1	36.36	24.24**
	Error	37	1.50	
	Total	47		
May 20, 1981	Block	2	11.60	44.62**
	Line/Hybrid	4	17.23	66.27**
	C1	1	1.94	7.46*
	C5	1	5.26	20.23**
	Error	23	0.26	
	Total	29		
June 8, 1981	Block	2	2.64	3.00
	Line/Hybrid	4	45.50	51.70**
	C6	1	0.07	0.08
	C2	1	8.87	10.08**
	Error	23	0.88	
	Total	29		

# Contrasts:
C1 = Regent vs Regent x Gullivar
C2 = Regent vs Regent x Schuster
C3 = Regent vs Regent x Kosa
C4 = Regent vs Regent x Bronowski
C5 = Regent vs Regent x Marnoo
C6 = Regent vs Regent x Karat

* Significant
** Highly significant