# HYBRID CANOLA BREEDING AT THE UNIVERSITY OF MANITOBA

P.B.E. McVetty, R. Scarth, S.R. Rimmer

Department of Plant Science, University of Manitoba,
Winnipeg, Manitoba, CANADA R3T 2N2

## INTRODUCTION

The University of Manitoba initiated a hybrid canola oilseed rape (B. napus L.) breeding program in 1980. All aspects of hybrid canola cultivar development have been studied, including pollination control system development and assessments of high parent heterosis, combining ability, pollination control system cost, male sterility inducing cytoplasm tolerance, blackleg disease resistance, and the assessment of agronomic performance of canola hybrids developed at the University of Manitoba.

## RESULTS AND DISCUSSION

### High Parent Heterosis

Initial studies at the University of Manitoba of high parent heterosis for yield, oil content and protein content involved hybrids derived from hand-produced crosses of spring habit European and Asian cultivars topcrossed to the Canadian spring canola cultivar Regent, grown at one location for one year (Sernyk and Stefansson 1983). Six topcross hybrids were tested, with the two best hybrids, Karat X Regent and Marnoo X Regent, yielding 43% and 38% more than the higher yielding parent Regent. The oil and protein contents of the Karat X Regent hybrid were not different from Regent while for the Marnoo X Regent hybrid, oil content was 0.8% higher and protein content 1.6% lower than for Regent. The results were sufficiently promising to justify the initiation of a hybrid canola breeding program at the University of Manitoba.

Further investigations of heterosis in inter-cultivar derived hybrids were undertaken at the University of Manitoba by Brandle and McVetty (1990). These authors reported significant levels of high parent heterosis for yield for the Karat X Regent and Marnoo X Regent hybrids, with heterosis levels averaging 20% for both hybrids in three trials grown over two years, confirming the preliminary report of Sernyk and Stefansson (1983). Several other hybrids were also tested and found to display significant levels of high parent heterosis for yield, (averaging 30%), for these intercultivar-derived hybrids. High parent heterosis for oil content occurred in only two of 21 hybrids tested while high parent heterosis of protein content did not occur in any of the 21 hybrids tested.

Studies of high parent heterosis in hybrids derived from crosses of inbred lines were also undertaken at the University of Manitoba by Brandle and McVetty (1989). Inbred lines were extracted from the cultivars Karat, Marnoo and Regent using conventional selfing to the $S_4$ level. These were evaluated in the field at two locations for two years to identify high, medium and low yielding inbred lines from each cultivar. These previously characterized inbred lines were then crossed in all possible combinations for the Karat X Regent and Marnoo X Regent hybrids. The 18 inbred-line derived hybrids were evaluated in the field in three trials grown over two years. The Karat X Regent inbred-line derived hybrids averaged 74.5% high parent heterosis for yield while the Marnoo X Regent inbred-line derived hybrids averaged 58.5% high parent heterosis for yield. The value of hybrids based on inbred lines compared to inter-cultivar derived hybrids is clear (74.5% versus 20% for Karat X Regent and 58.5% versus 20% for Marnoo X Regent). On the basis of this study, it appears that all hybrids

should be based on inbred line parents to maximize high parent heterosis levels for yield. There was no evidence of high parent heterosis for oil content or protein content in any of the inbred-line derived hybrids from either the Karat X Regent or Marnoo X Regent hybrids.

In a further study of 21 different hybrids produced using a half diallel cross of inbred lines derived from seven different cultivars, grown in three environments over two years, 19 of 21 hybrids displayed significant high parent heterosis for yield (Brandle 1989). No hybrids displayed high parent heterosis for either oil content or protein content.

Pollination Control Mechanism

The next phase of the research involved the development of a pollination control system to permit the production of commercial quantities of hybrid seed in the field. The University of Manitoba hybrid breeding program has concentrated on cytoplasmic male sterility (CMS) systems. Preliminary investigations looked at the nap, ogu and pol cytoplasms (Fan et al. 1986). The authors used 32 rapeseed cultivars from all over the world, making hybrids between these cultivars and male sterile plants containing one of the three male sterile cytoplasms listed above. All cultivars were found to be full maintainers for the ogu induced male sterility, and partial or full maintainers of the pol induced male sterility. Bronowski was a partial maintainer for the nap induced male sterility while the hybrid involving Lergo segregated for male sterility and male fertility, suggesting that Lergo is heterogeneous for genes conditioning maintenance and restoration of the nap induced male sterility.

Both the nap and pol cytoplasms offered the promise of development of a full workable CMS system for use in the field to produce commercial quantities of hybrid seed. There were indications from the literature, however, that the male sterility conditioned by both cytoplasms was temperature sensitive, and that male fertility reversion could occur if temperatures exceeded certain undefined levels. The temperatures at which male fertility reversion occurred were investigated by Fan and Stefansson (1986). It was found that nap plants were completely male sterile at temperature regimes of 22$^0$ C day / 16$^0$ C night, partially male sterile at 26$^0$ C / 20$^0$ C, and fully male fertile at 30$^0$ C / 24$^0$ C, while the pol plants were completely male sterile at temperature regimes of regimes of 22$^0$ C day / 16$^0$ C night, and at 26$^0$ C / 20$^0$ C, while they were partially male sterile at 30$^0$ C / 24$^0$ C. The temperature sensitivity of both the nap and pol male sterility inducing cytoplasms was recognized as being undesirable for use in the field to produce commercial hybrid seed. Plant to plant variation in the depth and stability of male sterility within the nap and pol open pollinated population materials suggested that improvements in male sterility could possibly be made.

The remaining component necessary to complete the above CMS systems were restorer sources. Fan et al. (1986) found no restorers for the ogu or pol cytoplasms among the 32 cultivars tested, while all 32 cultivars were restorers for the nap cytoplasm. Fertility in the pol cytoplasm was restored in the hybrid of a cross involving the pol oilseed rape (B. napus) cultivar Regent and the mustard (B. juncea) cultivar ZEM. Pure breeding restoration of the pol cytoplasm using the ZEM source occurred only in 2n = 40 addition lines. Further searching of the B. napus accessions at the University of Manitoba uncovered two genetically different pol CMS restorer sources, one the cultivar "Italy", and the other, the accession "UM2353" (Fang and McVetty 1989). Both restorer sources provided adequate male fertility restoration of the pol CMS system for commercial use in hybrid seed production.

The decision was made early in the 1980's to develop the pol CMS system as much as possible for use in the production of commercial quantities of hybrid seed. The primary problem was the development of pol CMS male sterile materials with adequate depth and stability of male

sterility. First generation pol CMS male sterile materials were based on cultivars, i.e. open pollinated populations. These populations had substantial plant to plant variation in degree of male sterility. Using a male sterility index rating scale of 0 to 6 where 0 identifies a male sterile plant in which no anthers produce any visible pollen, and where 6 identifies a male sterile plant in which each anther each produces any visible pollen, the variation in male sterility index rating in the pol CMS male sterile open pollinated populations was from 0 to 6 with a mean male sterility index rating of approximately 4.0. These results suggested that a switch to pure lines from populations would be required to improve the depth and stability of pol CMS male sterility. Burns et al. (1991) evaluated the male sterility of hybrids made using 24 $S_4$ inbred lines from each of the cultivars Karat, Lergo, Marnoo and Regent crossed to pol CMS male sterile plants of their respective cultivars. The open pollinated population derived female plants used were selected for maximum expression of male sterility prior to be used in the crosses. There were significant differences found in the maintenance capabilities of the inbred lines tested, indicating the presence of different maintainer genes among the inbred lines tested. The most promising maintainer lines were further backcrossed to their respective hybrids to generate backcross series for each cultivar. Deep and stable male sterility was obtained by backcross six for inbred lines from several of the above cultivars.

Heterosis Group Assignment

Heterotic patterns of geographic origin can be useful in the initial stages of a hybrid breeding program. Brandle and McVetty (1990) conducted a study involving seven open pollinated population cultivars of diverse geographic origin, four European, two Canadian and one Asian in origin, as parents, and their 21 derived hybrids, grown in three environments over two years to address this question. Results of this study indicated that, for yield, the Canadian and European cultivars belong to the same, or partially overlapping heterotic groups and that the Asian cultivar belonged to another. There were very few instances of significant heterosis for oil or protein content.

Combining Ability

An integral component of hybrid breeding in any crop is the assessment of general and specific combining ability. Brandle and McVetty (1989) used 18 hybrids derived from crosses of three inbred lines derived from each of three open pollinated population cultivars grown at three locations over two years and determined that general combining ability was much more important that specific combining ability. Further, there were found to be significant differences among inbred lines derived from the same open pollinated population cultivar, indicating the presence of variation for breeding value among related inbred lines. Additive genetic effects predominately influenced the expression of yield in this group of hybrids. This suggests that parents can be chosen directly on their agronomic performance.

Biological Cost of, and Tolerance to, the pol Cytoplasm

Cytoplasmic male sterility arises from imbalanced and negative, nuclear-cytoplasm interactions. These nuclear-cytoplasm interactions not only produce male sterility, but are also responsible for other associated negative effects on the agronomic performance of the genotypes present in the sterilizing cytoplasm. In a comparative study of three male fertility restored hybrids made in both the nap and pol male sterility inducing cytoplasms, conducted in five environments over two years, the hybrids in the pol cytoplasm were found to yield, on average, 23% less than the same hybrids in the nap cytoplasm (McVetty et al. 1990). The pol hybrids were also, on average, 3% lower in relative oil content than the nap hybrids, but not different for protein content. There was no evidence of high parent heterosis for oil content or protein content for either the nap or pol hybrids. The nap hybrids displayed, on average 70% high parent

heterosis for yield while the pol hybrids displayed on average, 31% high parent heterosis for yield. There is, therefore a biological cost associated with the use of the pol CMS system. The cost is not so high as to eliminate the heterotic advantage of hybrids made in this cytoplasm compared to the better parent used in the cross, so that, the pol cytoplasm, although not entirely desirable, can still be used to make hybrids until such time as a better alternative is found. McVetty et al. (1990) also reported that different hybrids displayed different biological costs of the pol cytoplasm. The Lergo X Regent hybrid displayed the least cost, i.e. the greatest tolerance to the pol cytoplasm, having only an 11% reduction in relative yield compared to the nap hybrid, a 1% reduction in relative oil content and no change in protein content. It seems, therefore, that different genotypes have different levels of tolerance for the deficiencies of the pol cytoplasm.

Blackleg Disease Resistance

The most serious disease of oilseed rape cultivars in western Canada is blackleg (Leptosphaeria maculans). Numerous potential parents for use in the hybrid breeding program have been assessed for their resistance to blackleg. The blackleg disease reaction of selected cultivars of interest is shown in Table 1.

Table 1. Blackleg disease resistance ratings for selected cultivars

| Canadian Cultivar | Blackleg Rating | European Cultivar | Blackleg Rating | Australian Cultivar | Blackleg Rating |
|---|---|---|---|---|---|
| Profit | MS[1] | Cresor | R | Maluka | R |
| Regent | S | Global | MR | Marnoo | MR |
| Stellar | MS | Karat | MS | Tatyoon | MR |
| Westar | HS | Lergo | MR | Wesbrook | MR |

[1] HS = highly susceptible, S = susceptible, MS = moderately susceptible, MR = moderately resistant, and R = resistant.

There was found to be substantial plant to plant variability for resistance to blackleg in all of the open pollinated population cultivars studied. This variability is reflected in the range of reaction to blackleg disease shown by the inbred lines derived from these open pollinated population cultivars, i.e. they occasionally range from HS to R within the inbred lines derived from one cultivar. The inbred lines chosen for use as parents in the hybrid breeding program must, therefore be individually screened for their blackleg reaction.

Hybrid Performance

The final determination of hybrid breeding program success is the performance of the material generated. In this regard, there have been incremental improvements in the performance of the hybrids bred at the University of Manitoba. The hybrid breeding effort has been focused on improving the performance of hybrids which were shown to be heterotic in the early 1980's, through male sterility enhancement, improved quality, improved oil and protein content, earlier maturity and improved disease resistance. The performance record of three selected canola hybrids evaluated during the 1980's is shown in Table 2.

Table 2. University of Manitoba pol CMS hybrid canola performance.

| Cultivar | Yield (% of Regent) | Oil % | Protein % | [Oil % + Protein %] |
|---|---|---|---|---|
| | 1986-87 (5 Station Years) | | | |
| Karat X Regent | 130[100][1] | 44.3 | 26.4 | 70.7 |
| Lergo X Regent | 144[100] | 44.8 | 25.8 | 70.6 |
| Marnoo X Regent | 120[100] | 43.6 | 26.0 | 69.6 |
| Regent | 100 | 45.0 | 25.1 | 70.1 |
| | 1.1 $t.ha^{-1}$ | | | |
| | 1988-89 (3 Station Years) | | | |
| Karat X Regent | 107[63] | 41.4 | 29.0 | 70.4 |
| Lergo X Regent | 123[75] | 42.4 | 28.2 | 70.6 |
| Marnoo X Regent | 126[79] | 41.9 | 27.5 | 69.4 |
| Regent | 100 | 42.1 | 28.0 | 70.1 |
| | 1.0 $t.ha^{-1}$ | | | |
| | 1990 (3 Station Years) | | | |
| Karat X Regent | 126[98] | 42.0 | 25.6 | 67.6 |
| Lergo X Regent | 134[95] | 42.6 | 25.6 | 68.2 |
| Marnoo X Regent | 117[98] | 40.6 | 26.5 | 67.1 |
| Regent | 100 | 42.8 | 25.6 | 68.4 |
| | 1.6 $t.ha^{-1}$ | | | |

[1] Percent hybridity for hand produced hybrid seed lots, 1986-87, and for field produced hybrid seed lots, 1988-89 and 1990.

Problems and Prospects

All breeding programs have a list of problems that need to be resolved in order to breed better cultivars. In the case of hybrid cultivars bred using the pol CMS system this list consists of the following:

a) male sterility improvement
b) cytoplasm tolerance improvement
c) oil content improvement
d) blackleg resistance improvement

a) There are now a limited number of adequately male sterile lines available to produce hybrid seed in the field using the pol CMS system. More pol CMS male sterile lines will be required to allow advancement in hybrid canola breeding. The development of these new pol CMS male sterile lines needs to be simplified, possibly through the use of the microspore-derived doubled haploid technique to rapidly produce pure maintainer lines.

b) There are significant differences in the degree of tolerance to the pol cytoplasm displayed by different nuclear genotypes. The available parental material needs to be screened for this tolerance, using crosses of potential parental lines to a pol CMS male sterile line with good male sterility. This may permit identification of parental lines which will produce hybrids with adequate performance in the pol cytoplasm.

c) The pol CMS hybrids tend to be low in oil content, compensating for this, in part, by being high in protein content. This problem may be

overcome through a combination of selection of parental lines which have high oil content and tolerance to the pol cytoplasm.
d) Blackleg resistance is conditioned by at least two dominant genes, so that hybrids will be blackleg resistant if at least one parent is pure breeding blackleg resistant. Some foreign cultivars have better blackleg resistance than Canadian cultivars. Screening for blackleg resistance must, however, become a routine part of the hybrid breeding effort.

## CONCLUSIONS

Hybrid canola cultivars promise substantial yield increases through utilization of heterosis. These yield increases have to be combined with maintained levels of oil and protein and improved blackleg resistance to meet Canadian canola cultivar needs. Meeting these objectives will be possible, but far from easy.

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