Final Report on the project:
Regulation of male sterility in canola for use in hybrid seed production
(June 2011)

Introduction and background:

The development of hybrids and hybrid seeds in crops is an important area in agriculture as the hybrids exhibit hybrid vigor i.e., heterosis, and are produced for desirable traits, e.g., early flowering, disease and abiotic stress resistance, and increases in crop yield. In many crops the hybrids are produced by hand emasculation of flowers which serve as the female parent for pollination with another line, the male (sperm) donor. The manual process of emasculation is labour-intensive and significantly increases the costs of hybrid seed production. As well, the hybrid seed produced by this method are not guaranteed with 100% hybridity.

The alternative methods include the use of natural or spontaneous male-sterile lines; genic (GMS) or cytoplasmic (CMS), the induction of male sterility by chemicals, i.e., gametocides, or by genetic engineering of crops for induction of male sterility. Each of these approaches has a merit as well as limitations and they have been used in different crops with varied success for hybrid seed production.

In the summer of 2000, we initiated a project with the help of Saskatchewan Canola Development commission - SCDC (formerly called CANODEV Research Inc.) to develop a non-transgenic, GMS system in canola (*Brassica napus*) in which fertility can be restored by chemicals. At that time we had isolated a line (3-8) in canola which was responsive to some plant growth substances (PGSs) in terms of fertility restoration in greenhouse conditions, but there was no information on the performance of the line in field conditions as well as its expression in subsequent generations.

Objectives:

1. To develop a GMS system using line 3-8 in canola in which fertility can be restored by PGSs and to generate 100% pure male-sterile seed for crossing with other elite lines to produce F_1 hybrids.
2. To test the female fertility of the GMS line in terms of seed and pod development.
3. To determine the performance of the GMS line 3-8 in field conditions in terms of fertility/sterility expression and to examine pollen development and germination.
4. To analyze the male sterility expression in the GMS line in subsequent generations from the pure ms seed produced by the PGS treatment in greenhouse and field conditions.

Research performed:

The first field trial of the line 3-8 in summer 2000 showed variation in the flower and stamen phenotype, particularly with regard to stamen length. Two separate lines 3-8H and 3-8D were identified; the former had short stamens whereas in the latter stamens were long but the petals had white streaks (Figure 1). However, neither of these lines produced viable pollen as tested by *in vitro* pollen germination on Hodgkin's medium. Thus, both these lines were functionally male sterile in the field.

Plants of 3-8 H and 3-8D were allowed to cross pollinate in the field by growing normal canola (Westar) plants alongside and there was good pod (silique) and seed set in male-sterile plants. The average number of seeds/pod was as follows: Westar = 32.26, 3-8H = 32.14, 3-8D = 38.31, indicating that female fertility is not affected in these lines.

In 2000- 2001 winter, 3-8H and 3-8D plants were grown in temperature- and light-controlled growth chambers for treating with PGSs to restore fertility in these two lines. However, the response to PGS treatment was variable in the two lines and 3-8 H plants were more responsive to the treatment than 3-8 D plants. In particular, line 3-8H3 (a sub-line of 3-8H) was most responsive to PGS treatment i.e., it produced normal anther and pollen (Figure 2 arrow) and the pollen were viable, i.e., when used for self-pollination they resulted in pod and seed development. Seeds were collected from these plants and the future work was focused on line 3-8H3.

For the next 3 summers (2001 to 2004), 3-8H3 plants were grown in the field from the seed produced by PGS treatments. Unfortunately, there was variation in the flower and stamen phenotype of plants in the next two generations. In fact both male-sterile and male-fertile plants were identified in the field, although the percentage of male-sterile plants was higher (> 50%) than the male-fertile plants. Some of the 3-8H3 male-sterile plants were collected from the field and brought to the greenhouse for further treatment with PGS and the seed thus produced were grown in summer 2005. There was some variation in pod and seed development in plants and plants were further segregated into two lines; 3-8H3-6 and 3-8H3-8, and 3-8H3-6 (now called H3-6) was selected for further work.

In 2006, H3-6 plants were grown in the greenhouse and in the field and none of the plants showed the normal phenotype, i.e., they were all male-sterile. However, there was variation in flower morphology and stamen development and the following three phenotypes were recognized (Figure 3):

1. Flowers with short stamens and no pollen (SS)
2. Flowers with long stamens and no pollen (LS)
3. Flowers with long stamens with shrunken anthers and some pollen (LSS)

In the field conditions, the relative distribution of plants with the above three phenotypes was: SS = 74%, LS = 24% and LSS = 2 %. The phenotype of SS flowers with short, i.e., invisible stamens, was ideal for cross pollination for hybrid seed production (Figure 4, arrows indicate the SS phenotype). There was no pollen produced in plants with SS and LS flowers, but some pollen was present on the shrunken anthers of LSS flowers and *in vitro* germination tests showed some germination indicating that not all plants in the field were completely male-sterile. We then isolated further lines of H3-6 in the field i.e., H3-6-1, H3-6-2, H3-6-6, H3-6-9, H3-6-11, H3-6-12 and H3-6-19, and of these H3-6-2 (line 6-2), H3-6-11 (line 6-11) and H3-6-19 (line 6-19) showed good pod and seed set in open pollination in the field (Figure 5).

Plants of the three lines 6-2, 6-11 and 6-19 were grown in the greenhouse and treated with PGS and line 6-19 showed the least variability in the phenotype, and best response for fertility restoration. Thus, in summer 2007 and 2008, seeds from self-pollinated plants of the line 6-19 were grown in the field and plants were examined carefully for male sterility expression. Unfortunately, in the next generation there was variability in line 6-19 in the field both in terms of the phenotype i.e., all three types of flowers SS. LS and LSS were produced. And, in some plants the expression of sterility was not stable, i.e., plants initially produced male-sterile flowers but later flowers were normal-looking with long stamens and anthers had some pollen.

Concluding remarks:

Our work with the GMS canola lines has shown that there is variability in the expression of sterility both between plants and on the same plant in different lines that we tested. This was specially the case in field conditions in comparison to growth chamber or greenhouse conditions. In general, majority of plants of the lines that we tested were with short stamens and were completely male-sterile, but those with long stamens either had no pollen produced while others produced some pollen which when tested under *in vitro* conditions showed germination. Thus, at this time we are unable to report on a GMS line in canola which in the field conditions would show 100% male sterility.

On the positive side, GMS plants with long stamens and no pollen respond very well to our PGS treatment in terms of fertility restoration. And, the female fertility of these male-sterile plants is as good, if not better, than the normal Westar plants. Thus, whereas we are able to generate male-sterile seed from PGS-treated plants, their expression in the field is varied and therefore, unfortunately at this time we are unable to recommend this system for large scale hybrid seed production in canola. Future work focused especially on plants with SS phenotype (Figure 4) may lead to isolation of a line which is stable in field conditions.