My Personal Hybrid Canola History

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Quick chronology of my work on hybrid canola

- **1978 Started PhD with Dr Baldur Stefansson at the University of Manitoba, Winnipeg, Manitoba Canada on hybrid canola development**
- **1982 Completed PhD and was hired by University of Manitoba to continue working on hybrid canola development**
- **1984 Accepted an offer from Allelix Inc of Mississauga Ontario Canada to work on hybrid canola development**
- **1986 Moved to a hybrid canola breeding position at Conti Seeds in southern Manitoba Canada to work on hybrid canola development**
- 1989 Accepted a position with Agrigenetics Inc (Lubrizol) in Madison WI USA to work on high stability vegetable oil development for use by Lubrizol as environmentally friendly lubricants (multiple crops: canola, sunflower, safflower, peanut, minor oilseeds such as Cuphea, Meadowfoam, Lesquerella, Vernonia) – NO LONGER working on hybrid canola development; Agrigenetics eventually became part of Mycogen Corp which eventually became Dow AgroSciences)

This history focuses on my experiences from 1978 through 1988 when I was working on hybrid canola development

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In 1978 when I completed my BSc Ag in Plant Science at the University of Manitoba, I was interested in going on to do a PhD. I talked to Anna Storgaard about who would be a good professor to do a PhD with at the University of Manitoba. I was originally thinking about doing a PhD in horticultural plant breeding working on crops like trees, shrubs and flowers. She said well that's okay but I'd probably have trouble getting a job as there was only one PhD horticultural plant breeding position in the province and they had just filled that position with a young person. She suggested go talk to Dr. Baldur Stefansson about an exciting project in canola breeding (I knew pretty much nothing about canola at that time. As everybody should know, Dr. Stefansson was the one of the two fathers of canola, the other being Keith Downey who was with Agriculture Canada in Saskatoon. She told me that I would never regret working with Dr Stefansson on canola and she was right on.

I took Professor Storgaard's advice and went and talked with Dr Stefansson. He said that he was looking for someone to do a PhD with him on a really hot topic area which was hybrid Brassica napus spring canola development, a new concept because all varieties to date had been developed by open-pollinated pedigreed selection. Dr Stefansson had obtained seed of spring Brassica napus from Dr Toshio Shiga in Japan of what he called the nap CMS system. This was very exciting as it could potentially enable the development of hybrid spring B. napus canola. I said that this sounded like a great area to work on and I said let's get started. And launched my career in hybrid canola breeding.

Dr Stefansson and I discussed how to approach this exciting area of research and decided that I would investigate the potential for hybrids in spring Brassica napus by evaluating the following:

1. A pollination control system (cytoplasmic male sterility) for making hybrid seed of spring B. napus
2. Determination if there was sufficient hybrid vigor in spring B. napus to justify the development of hybrids versus the current open-pollinated cultivar development approach being used to develop new varieties

Based on what my undergraduate training in plant breeding had taught me, it seemed counterintuitive that a naturally self-pollinating crop like B. napus would show much hybrid vigor but keeping an open mind we said let's investigate the amount of heterosis in spring Brassica napus.

So my work at the University of Manitoba would involve evaluating these two requirements for successful hybrid breeding of spring B. napus:

- a workable pollination control system (we were focused on CMS systems, the nap CMS system in particular) and
- demonstration of sufficient heterosis in spring B. napus by producing hand crossed hybrid seed and evaluating the performance of the hybrids against the parent lines used to make the hybrids

My initial experience with the nap CMS system that Dr. Stefasson had obtained from Dr Shiga in Japan resulted in the following findings:

1. The flower petals on the nap CMS line that we received from Shiga were smaller and the anthers were lower down on the flowers than in regular Brassica napus varieties and anthers produced very limited if any pollen.
2. Genetic analysis of the cytoplasm by other researchers showed that the nap (napus) cytoplasm was cytoplasm that is present in the vast majority of spring Brassica napus cultivars but those cultivars also had nuclear genes that restored male fertility.
3. There were however some cultivars such as Bronowski and Lergo which when crossed into the nap cytoplasm resulted in male sterility. These cultivars had the cam (campestris) cytoplasm based on genetic analysis of their cytoplasmic organelles and were fully male fertile in the cam cytoplasm.

As mentioned the petals were smaller, the anthers were lower down in the flowers and there was very little pollen produced in the anthers under some conditions. The male sterility was best under cooler conditions but when the temperatures were elevated during flowering, the male sterility would break down and there would be more pollen production thereby resulting in a lot of self-pollination on the nap CMS-lines.

Based on these observations, I came up with some ideas for how this system might be used. We could grow the A-line parents of hybrids under warm conditions and they would just naturally self-pollinate thereby increasing seed of the A-line parent without the need for a B-line maintainer. Then we could produce hybrid seed in reliably cooler conditions under which the A-line would be male sterile thereby pollination would be from the adjacent R-line (restorer) parents. This however, was not viewed as a very desirable because it's hard to get reliably cool conditions for producing high hybridity hybrid seed of the quantity needed for planting millions of acres of hybrid canola.

I did do some additional work to try to improve the male sterility in the nap cytoplasm by using maintainer genotypes like Bronowski and Lergo but I still had the same issue with the sterility breaking down under high temperatures during flowering.

A strength of the nap CMS system is that breeding R-line male parents was very straightforward as most Brassica napas cultivars had the nap cytoplasm and thus were restorers. Breeding programs would be able to focus on developing A-lines with improved and more stable male sterility and their corresponding B-line maintainers and these A-lines could be crossed with just about any spring Brassica napus cultivar (with nap cytoplasm) to make hybrids. This is like what Greg Buzza at Pacific Seeds in Australia found with the Polima system (pol cytoplasm). It was challenging to develop A-lines with stable male sterility and corresponding B-lines but then those good A-lines could be crossed with just about any other spring nap cytoplasm Brassica napus cultivar to make hybrids with full male fertility. Male sterility in pol CMS A-lines is much superior to that of nap CMS A-lines.

The other component of my research at the University of Manitoba involved testing of a number of spring Brassica napus hybrids to determine the amount of heterosis in spring Brassica napus. I selected Regent as one parent of all the hybrids I would evaluate as it was a widely grown well adapted cultivar developed by Dr. Stefansson. It had good yields, good disease tolerance and good agronomics and was widely grown in western Canada.

I selected a diverse range of cultivars to cross with Regent as in the table at right. A couple of these, Karat and Marnoo, were canola quality.

The seed of these hybrids were all made by hand-pollination in the greenhouse. I also bagged plants of the parent cultivars to produce check seed for the field trials. The trials were conducted over the 1980 and 1981 field seasons.

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

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

†Canola quality cultivars.

The results are provided in the following tables:

Table 2. Agronomic, yield and quality data for the hybrid summer 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)	Total dry matter yield (kg/ha)	Apparent harvest index (%)	Thousand-seed weight (gm)	Oil‡ (%)	Protein‡ (%)
21 May, 1980	Regent	14	37	95	116	2.2	2198	7691	28.4	3.25	44.3	27.9
	$(GXR)F_1$	13	38	97**	131**	1.8	3072**	9952**	30.9*	3.30	44.1	27.4
	Gullivar	14	42	101	134	1.7	2272	8751	26.2	3.20	43.4	27.1
	$(SXR)F_1$	14	40**	98**	133**	1.8	2525	8991*	28.1	2.78**	45.8**	25.4**
	Schuster	15	46	102	140	2.7	1899	8367	22.4	2.47	43.2	25.7
	$(KXR)F_1$	14	38	95	129**	1.8	2355	8317	28.2	3.20	43.5	26.6**
	Kosa	14	41	96	137	2.3	2198	7701	28.5	3.00	41.7	26.3
	$(BXR)F_1$	15	40**	97**	133**	1.0**	2160	8476	25.4*	2.75**	45.7**	26.7**
	Bronowski	16	48	100	140	1.3	1512	8620	18.0	2.00	44.9	25.9
	CV¶ (%)	14.0	3.1	1.9	3.8	23.9	15.6	11.4	8.1	6.4	1.7	1.9
20 May, 1981	Regent	12	36	81	104	3.0	1529	6156	24.8	3.26	45.1	27.6
	$(GXR)F_1$	10**	37**	83**	113**	3.0	2187**	7872**	27.8*	3.17	44.3*	26.8*
	Gullivar	11	40	85	115	3.0	1130	6491	17.4	3.45	39.5	31.1
	$(MXR)F_1$	11*	37**	82*	111**	3.0	2106**	7706**	27.2*	3.18	45.9*	26.0**
	Marnoo	11	41	85	112	3.0	1484	6702	22.2	3.15	42.9	29.0
	CV (%)	5.9	1.6	1.0	4.0	0.0	15.6	9.3	8.9	3.4	1.5	2.2
8 June, 1981	Regent	5	35	83	97	2.8	1082	5064	21.0	3.35	43.8	29.1
	$(KXR)F_1$	5	36	84	109**	2.3	1545**	6512**	23.9	2.99**	44.0	28.7
	Karat	7	41	88	91	1.0	908	6273	14.7	3.09	42.4	30.7
	$(SXR)F_1$	5	38**	86**	109**	2.8	1277	5661	22.5	2.99**	43.9	27.2**
	Schuster	5	50	99	98	1.3	978	6569	15.0	2.81	42.9	25.7
	CV (%)	4.6	2.8	1.1	5.7	25.9	20.9	14.7	16.3	4.6	1.5	2.8

†From emergence.
‡Percent of seed dry weight.
§1 = no lodging; 5 = completely lodged.
¶Coefficient of variability.
*F_1 values significantly different from values for Regent; **F_1 values highly significantly different from values for Regent.

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

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

As can be seen in these tables, several of the hybrids (Gullivar x Regent, Marnoo x Regent and Karat x Regent) showed significant heterosis for seed yield as a % of Regent, the other parent and the midparent.

The full results of these trials were published in Sernyk, J. L. and Stefansson, B. R. 1983. Heterosis in summer rape (Brassica napus L.) Can. J. Plant Sci. 63: 407-413.). A copy of the paper is provided.

There was one paper that stood out to me about hybrid vigor in spring Brassica napus. This was a study done by Agriculture Canada Saskatoon where they made di-haploid lines via anther culture from I believe Westar. This study was to determine that if you do severe inbreeding in Brassica napus (i.e. producing di-haploids), do you reduce the yield potential of the DH lines that are produced. I can’t find a copy of the paper but I recall they found that some of the di-haploid lines actually exceeded the yields of the variety (Westar) per se, which suggested that any heterosis in the open pollinated variety Westar could be fixed.

As part of my PhD research at the University of Manitoba, I also did some crosses to move a dominant white flower color from Raphanus sativus to Brassica napus. I thought this white flower color could be incorporated into the R-lines of a hybrids and based on the flower color of the hybrid progeny plants, it would show how successful the pollination control was working in the production of the hybrid seed (it would be expected to be 100% white flowered if pollination control was complete). It was relatively easy to transfer this trait as it was easy to identify the dominant white flower colored progeny and backcrossing to yellow flowered Brassica napus line. However, I observed some unusual transmission rates for the white flower color of the transferred trait through the male and female gametes. What I found was that the white flower color was transmitted at less than the expected 50% rate from the heterozygous condition. Genetically I found that transmission from white flowered heterozygous plants was reduced to just 24.3% in female gametes and 22.1%,16.7% and 0.7% through male gametes as in the table below. As I mentioned transmission was expected to be 50% through both the female and male games from the heterozygous condition if normal Mendelian genetics was occurring.

TABLE II

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	$F_1 \times Y$ (bud and delayed)	24.3
Male	$F_1 \times F_1$ (bagging)	22.1
Male	$Y \times F_1$ (bud)	16.7
Male	$Y \times F_1$ (delayed)	0.7

To help better understand what was going on, I did microscope investigation of meiosis and found that the white flower color was on a small alien chromosome from the radish which tended to get lost when being transmitted to gametes from the heterozygous condition. These results concurred with what had been found by for wheat monosomic addition lines. This suggested that the Ogura Radish restorer gene would need to be incorporated into a Brassica napus chromosome to get normal Mendelian inheritance. A full copy of this research paper is included *Sernyk, J L and Stefansson, B R 1982 White Flower Color in Rape (Brassica napus) Associated with a Radish (Raphanus sativus) Chromosome. Can. J. Genet. Cytol. 24 729-734*.

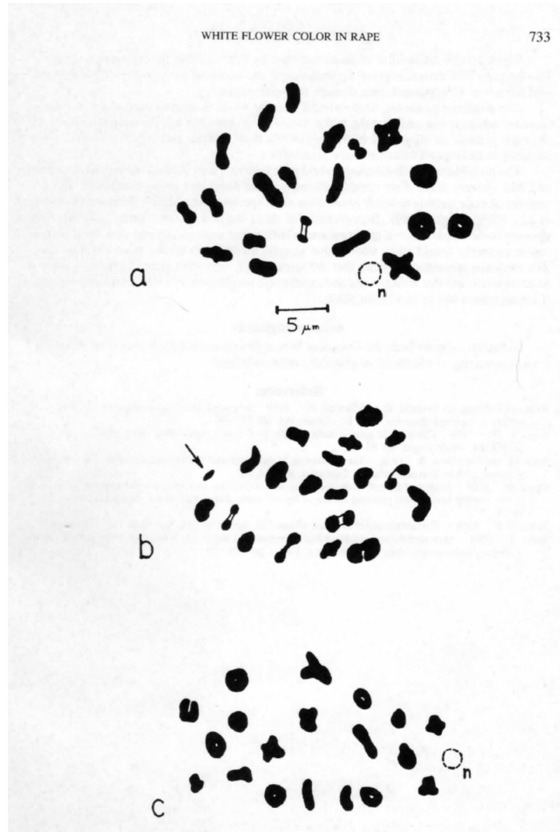

Fig. 1. PMCs of *B. napus*: (a) white flowering strain — 20 II, (b) white flowering F_1 — 19 II + 1I (univalent is indicated with arrow), and (c) yellow flowering strain — 19 II. Nucleoli are also indicated (n).

I finished my PhD in 1982 and the University of Manitoba offered me a position in hybrid spring B. napus development and teaching and training new graduate students in plant breeding. There were several additional graduate students working on Canola breeding at the University of Manitoba, some of them with me and some of them with Dr. Stefansson and Dr. McVetty looking at other species related to Brassica napus to explore the development of additional CMS systems. This included Diplotaxis muralis which required making wide intergeneric crosses and then backcrossing to B. napus. and the Ogura CMS system. Dr Stefansson had acquired seed of Brassica napus in the Ogura cytoplasm but we did not have access to a restorer; these lines had excellent male sterility but had chlorosis issues and the flowers had limited nectar production. Researchers at other organizations went on to successfully make hybrids with the radish CMS system correcting the nectar production issue and transferring male fertility restorer genes from the radish.

That basically summarizes what I did at the University of Manitoba. In 1984 I took a position to work for Allelix Inc in Mississauga Ontario to work on hybrids and in 1986 I accepted a position with Conti Seed in Winnipeg Manitoba (aka Pacific Seeds in Australia) working with Greg Buzza. I worked in western Canada to help evaluate Pacific Seeds Polima hybrids. I had a breeding program to develop new parent lines using the Polima CMA system. Greg Buzza has covered this really well his summary of the Polima CMA system.

One thing I remember about the Polima CSM system is it the A-lines also had the reduced petal sizes and when we were making the hybrid seed on the female parents there tended to be reduced seed set because the honey bees found a way to thieve the nectar and the pollen without actually pollinating the stigmas on the female parent lines. This resulted in extremely large hybrid seed. I remember Hyola 401 had seed that was like small peas in size. I think some of the yield advantage of the polima hybrids back then was due to just those larger seeds which allowed the seedlings to pop out of the ground faster and be more tolerant to flea beetle damage and get a head start in the growing season. Pacific Seeds' Polima based hybrids were also day-length neutral (i.e. they did not need long days to flower) so they would flower earlier which gave them an advantage in Western Canada. The issue of pollinating the Polima A-line flowers was solved to a large degree by switching from honey bee pollinators to leaf cutter bee pollinators which just crawled across the stigmas on the A-lines. Again, Greg Buzza has covered this really well so I'm not going to repeat that here.

After Conti seed, I went to work for Agrigenetics/Lubrizol on specialty oils in multiple crops. I wasn't working on hybrid development per se throughout the rest of my career. We focused on making high oleic/low linolenic acid canola varieties for Lubrizol to use to produce environmentally friendly lubricants/hydraulic fluids. The program was purchased by Mycogen and ultimately when the program was bought by Dow Agrosciences, David Dzisiak realized that these high stability fatty acid profiles (Omega-9 canola and sunflower) were also ideal for nutritional purposes too and that's when the Omega-9 oil programs really took off.

Throughout much of my career beginning as a graduate student and working as a canola breeder, I started keeping a *Catalogue of Oilseed Rape Cultivars compiled by Larry Sernyk*. I intended it to be a reference for breeders and researchers to use to understand the pedigrees of spring and winter, Brassica napus and Brassica rapa cultivars by country. It was easy to do this early on because much of the breeding was by public research institutions. With the advent of hybrids, private organizations got involved in oilseed rape breeding which made it very difficult/impossible to get the pedigrees of the varieties/hybrids. I used to get frequent requests for copies of the catalogue but requests have subsided in recent years. A copy of the last update which was made in 2003 when I was working with Dow AgroSciences is attached. I tried to get the Canola Council of Canada to take on updating this catalogue but was not successful. Lists of approved cultivars are still available (annually updated) from the variety registration authorities in the individual countries where oilseed rape is grown.