Brassica seed quality breeding at the University of Manitoba

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Abstract

University of Manitoba canola/rapeseed breeding programs have always focused on improving seed quality with increases in oil, protein and sum of oil and protein, reduction in fibre and oil composition modifications targeted. Genomics has recently been added to the wide range of techniques used at the UM to develop high seed quality canola/rapeseed.

Key words: oil, protein, oil composition modifications, fibre, canola, rapeseed

Canola/rapeseed is the major oilseed crop grown in Canada. In addition to continued improvements of agronomic performance, steady improvements in the seed quality of canola/rapeseed varieties are required to maintain the competitive edge of *Brassica* varieties compared to other oilseeds. Seed quality in canola/rapeseed is multi-faceted, consisting mainly of oil, protein, glucosinolates and fibre levels in the seed and oil composition.

The University of Manitoba (UM) canola/rapeseed breeding programs monitor and select for oil and protein levels and for the sum of oil and protein levels in the seed. The maximum oil, protein and sum of oil protein contents in *B. napus* observed in yield trials in recent years at the UM are shown in Table 1. All of these materials possess black seeds. Compared to the checks, it appears that oil level in black seeded *B. napus* lines can be increased by approximately 5%, protein level by over 1% and the sum of oil and protein level by over 7% in black seeded lines and likely by even more in yellow seeded lines.

Table1. Maximum oil, protein and sum of oil and protein levels in *B. napus* from yield trials grown at the UM 2002-2006

Line Type / I.D.	Pedigree	Oil[1] %	Protein[2]%	Sum (O+P)[3] %
Canola (Oil max)	96LL112×HiQ	51.0	45.3	96.3
Canola (Pro max)	97LL105×HiQ	50.8	46.3	97.1
Canola (Sum max)	97LL105×HiQ	50.8	46.3	97.1
Low Lin (Oil max)	Polo×92-588	52.9	47.8	100.7
Low Lin (Pro max)	970170×97C49	49.8	50.8	100.4
Low Lin (Sum max)	99LL120	52.3	50.0	102.3
HSO (Oil max)	970126×97C49	53.0	45.6	98.5
HSO (Pro max)	970126×97C49	51.8	48.3	100.1
HSO (Sum max)	970126×97C49	51.8	48.3	100.1
HEAR (Oil max)	HR100×Sponsor	54.8	42.5	97.3
HEAR (Pro max)	HR100×Bianca II	50.7	48.3	99.0
HEAR (Sum max)	HR100×Bianca II	52.5	46.8	99.3
Check (Oil max)	Q2	49.7	43.4	93.1
Check (Pro max)	46A65	45.6	49.4	95.0
Check (Sum max)	46A65	45.6	49.4	95.0

[1,2,3] Oil, protein and sum (O+P) @ 0% H_2O

Molecular marker assisted selection for increased oil concentration using the Sequence Related Amplified Polymorphism (SRAP) marker system (Li and Quiros 2001) has begun recently at the UM. Large DH line populations are being developed and evaluated for oil level variation currently.

Reduced fibre content in canola/rapeseed is a function of seed coat thickness, which is seed-coat-colour related. The yellow seed coat colour trait in *Brassica* significantly reduces fibre content. UM research and development on yellow seeded canola/rapeseed currently focuses on the development of SRAP molecular markers for the genes conferring the yellow seed coat trait in *Brassica rapa* and *Brassica napus*.

Oil composition modification research continues to be a focus of the UM *Brassica* breeding programs. Reduction in saturate level has been pursued using microspore mutagenesis, intraspecific crosses within *B. rapa* and *B. napus*, interspecific crosses and DH line production from the $BC1F_1$ plants of these crosses (McVetty and Scarth, 2002). Low saturated fat DH *B. rapa* lines from the cultivar Reward were created using microspore mutagenesis at the Plant Biotechnology Institute at

Saskatoon, SK. These mutant DH lines were crossed to normal DH lines from the cultivar Reward and then DH lines produced from the F_1 plants. These *B. rapa* DH lines were then crossed to selected *B. napus* genotypes which were low in saturated fat levels to create interspecific F_1's. The production of DH lines from the interspecific F_1's was difficult, so a backcross to the *B. napus* genotypes was done. Selection for low saturate level within the high stability oil (HSO) *B. napus* materials developed at the UM was also done. The saturated fat levels achieved in selected materials developed in this research are shown in Table 2.

Table 2. Saturated fat levels in selected *B. rapa, B. napus* and *B. napus* × *B. rapa* cross progeny grown at the UM 2002-2006

Line Type / I.D.	Pedigree	Origin	Saturated fat %
B. rapa			
MDHFA 16-4	Reward DH line	Microspore mutagenesis at PBI	5.3
MDHFA 18-1	Reward DH line	Microspore mutagenesis at PBI	5.8
MDHFA 105-23	Reward DH line	Microspore mutagenesis at PBI	4.5
DH14-146 × MDH105-23	Reward	Intraspecific cross	5.1
DH14-146 × MDH105-23	Reward	Intraspecific cross	4.9
DH14-146 × MDH105-23	Reward	Intraspecific cross	5.2
B. napus × *B. rapa*			
1OOGHDH	Dynamite × MDHFA 18-1	Interspecific cross $BC1F_1$ DH line	6.5
4OOGHDH	Dynamite × MDHFA 18-1	Interspecific cross $BC1F_1$ DH line	6.2
21000GHDH	HSO 970247 × MDHFA 16-4	Interspecific cross $BC1F_1$ DH line	6.6
B. napus			
HSO DH 940	HSO 970073×HSO 97C105	Intraspecific cross DH line	5.5
HSO DH 945	HSO 970001 × HSO 97C29	Intraspecific cross DH line	5.6
HSO DH 989	HSO 970170 × HSO 97C121	Intraspecific cross DH line	4.8
HSO DH 1007	HSO 970001 × HSO 97C29	Intraspecific cross DH line	5.6

The reduction in linolenic fatty acid level with resulting increases in oleic level in canola oil types, has been a long term breeding objective at the UM (McVetty and Scarth 2002). The initial focus was on the reduction of linolenic acid, using the M11 mutant line from Rakow (1973), with the cultivars Stellar (1987), Apollo (1993) and Allons (1994), having 3%, 1.7% and 2.5% linolenic acid, respectively, released by the UM. Linolenic acid level in *B. napus* is controlled by two genetic loci with additive effect. These loci have been co-located to two fad3 genes in *B. napus* by genetic mapping. Minor genes, maternal and cytoplasmic effects have also been associated with the variation in the linolenic level in *B. napus*. As a result, linolenic acid level generally shows continuous variation in crosses between high and low linolenic lines. The complete elimination of linolenic acid from the seed oil is not likely to be achieved by conventional breeding, as linolenic acid plays an essential role for normal plant growth and reproduction.

Oleic acid concentration in Stellar, Apollo and Allons ranged from 60 to 66%. Comparative studies showed that oils with relatively higher oleic and lower linolenic levels than conventional canola oil possess an improved oxidative stability without the requirement of partial hydrogenation, and produce less undesirable products during deep frying (Warner and Mounts 1993). Mid to high oleic acid oils have equivalent heat stability to saturated fats and are suitable replacements for them in commercial food service applications that require long life stability. The profile of the optimum high stability oil (HSO) based on these comparisons has a 67 to 75% oleic acid level compared to conventional canola cultivars with about 61% oleic acid in the seed oil. The low linolenic canola breeding program at the UM has placed increasing emphasis on oleic acid concentration in addition to linolenic acid concentration, to create HSO materials. The minimum linolenic acid % and maximum oleic acid % in these HSO materials are shown in Table 3.

Table 3. Oleic acid level and linolenic acid level in high stability oil (HSO) *B. napus* grown in yield trails at the UM 2002-2006

Line type / I.D.	Pedigree	Origin	Oleic acid %	Linolenic acid %
B. napus×*B. napus*				
LL canola×LL canola (linolenic min)	970073×97C49	DH line	75.4	1.4
LL canola×LL canola (oleic max)	970126×97C49	DH line	76.6	1.5
B. napus×*B. rapa*				
LL canola×low sat *B. rapa* (linolenic min)	(970247×FA16-4)×970247	Interspecfic cross DH line	76.0	2.1
LL canola×low sat *B. rapa* (oleic max)	970247 × FA16-4	Interspecfic cross DH line	76.0	2.1

The development of industrial end use high erucic acid rapeseed (HEAR) varieties with increased erucic acid level has been a major breeding objective at the UM. A Swedish origin rapeseed with high erucic acid level was crossed to Tower to create the first HEAR variety, Reston (1982). A number of HEAR cultivars have been released by the UM after Reston including Hero (1989), Mercury (1992), Neptune (1995), Venus (1995), Castor, (1996), Millennium 01 (1998), Millennium 02

(1999) and Millennium 03 (1999). More recently, two new Roundup Ready HEAR cultivars, Red River 1826 (2006) and Red River 1852 (2006) have been released. Erucic acid level in *B. napus* is controlled by two genetic loci with additive effect. Minor genes have also been associated with variation in erucic acid level so that erucic acid level shows near continuous variation from zero to over 60% in canola×HEAR crosses. Erucic acid level has increased from 45% in Reston to 55% in the MillenniUM series HEAR cultivars. The Roundup Ready Red River HEAR cultivars have an erucic acid concentration of approximately 53%. The lower level of erucic acid in the Red River HEAR varieties is a minor gene effect not related to the Roundup Ready gene or to Roundup tolerance. The maximum erucic acid level of HEAR varieties grown at the UM in recent years are shown in Table 4.

Table 4. Erucic acid level in *B. napus* grown in HEAR yield trails at the UM 2004-2006

Line type / I.D.	Pedigree	Origin	Erucic acid %
B. napus×*B. napus*			
HEAR×HEAR (erucic max)	HR100×HR200	OPP	56.7
HEAR×Canola (erucic max)	HR 100×Bianca II	OPP	56.4
HEAR×RR Canola(erucic max)	HR499×Kelsey RR	OPP	55.9

The development of super high erucic acid rapeseed (SHEAR) germplasm with erucic acid levels of greater than 66% is also an important rapeseed breeding objective at the UM. This goal has been approached by re-synthesizing *B. napus* through crossing selected lines of the two ancestral diploids, *B. rapa* and *B. oleracea*, which can incorporate C22:1 into the Sn-2 position, (Taylor et al. 1995), followed by chromosome doubling. Re-synthesized *B. napus* plants accumulate levels of C22:1 over 60%. SHEAR development is currently being pursued using two approaches, 1) in-house microspore mutagenesis of resynthesized *B. napus* lines and 2) fatty acid biosynthesis transgene pyramiding in collaboration with the Plant Biotechnology Institute. The PBI transgenes affect fatty acid biosynthesis pathways and influence erucic acid levels in the oil. The maximum erucic acid concentrations observed in the SHEAR materials are closely approaching the theoretical upper limit of 66% erucic acid (Table 5).

The preservation of seed quality through incorporation of disease resistance is another important breeding objective, with SRAP molecular marker assisted disease resistance gene pyramiding research and development in progress. Molecular markers for several blackleg disease resistance genes will soon be available for use in the UM canola/rapeseed breeding programs.

Recent breeding efforts involve the development of herbicide tolerant canola/rapeseed cultivars and the development of hybrid canola/rapeseed cultivars. The UM has developed several canola varieties using the OXY gene conferring tolerance to the herbicide bromoxynil, including Armor BX (2000), Cartier BX (2000), 295 BX (2000), Zodiac BX (2000) and Renegade BX (2001).

Hybrid canola/rapeseed variety development is increasingly important at the UM with parental line combinations which maintain or enhance seed quality in the hybrids being identified.

Table 5. Maximum erucic acid level in *B. napus* grown in SHEAR yield trials or confined field trails at the UM 2003-2006

Line type / I.D.	Pedigree	Origin	Erucic acid %
B. napus×*B. napus*			
SHEAR×SHEAR	S14-24-158×S69-6-10	Resynthesized *B. napus* OPP cross	64.3
SHEAR× PBI TG1	S02R2668×HR696 pSE	Resynthesized. *B. napus* OPP×oleic to linoleic block transgene (PBI)	63.1
SHEAR×PBI TG2	S02R2709×NP00-3094	Resynthesized. *B. napus* OPP×FAE1 transgene (PBI)	62.6
SHEAR×PBI TG3	S02R2668×NP01-0649	Resynthesized. *B. napus* OPP×sn-2 transgene (PBI)	63.1

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