

Analysis of FAD2B alleles in interspecific derived *Brassica juncea* and *Brassica carinata*

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INTRODUCTION

Oriental mustard (*Brassica juncea* L.) is predominately grown as a condiment in western Canada and is characterized by its high allyl (2-propenyl) glucosinolate content, intermediate erucic acid and low oleic acid content (OAC). Condiment mustard oil typically contains 10-15% oleic acid (18:1 c9), 20-25% linoleic acid (18:2 c9, c12), 14-16% linolenic acid (18:3 c9, c12, c15) and 25-30% erucic acid (22:1 c13). Due to the health concerns surrounding erucic acid, zero erucic acid *B. juncea* forms were created (Kirk & Oram 1981). These lines have altered fatty acid elongase (*FAE*) genes (Kanrar et al. 2006) which prevent very long chain fatty acids from being produced.

The first low glucosinolate *B. juncea* breeding lines were created via an interspecific cross with *B. rapa* (Love et al. 1990). Initial *B. juncea* breeding lines had either a favourable fatty acid or glucosinolate profile. Additional crossing led to the coupling of these two traits, leading to canola quality *B. juncea* material (Potts et al. 1999).

Current registration requirements for canola quality *B. juncea* cultivars in Canada include an oleic acid content of not less than 55%, erucic acid levels less than 2% and total glucosinolates not more than 12 μ moles per gram of whole seed at 8.5% moisture. The first commercial cultivars were released in 2002 by Viterra (formerly Saskatchewan Wheat Pool). Due to the heavy selection pressure applied to breeding material in order to fix the fat and meal profiles, genetic variation is relatively low in *B. juncea* canola (Burton et al. 2004) and further work is therefore required to improve the agronomic performance of new strains.

The Δ -12 oleate desaturase (*FAD2*) gene is responsible for converting oleic acid to linoleic acid in the endoplasmic reticulum of plant cells through the introduction of a double bond on the twelfth carbon of the fatty acid chain (Murphy and Piffanelli 1998). Inheritance studies in *B. napus* high oleic mutants indicated that one major gene controls OAC in the seed and other genes are responsible for fatty acid composition in leaves and roots (Schierholt et al. 2001). Recent QTL analysis has located one region responsible for 76.3% of the variation present in a *B. napus* population (Hu et al. 2006). This region was mapped to chromosome N5 and has been associated with the *FAD2* gene. Work in *B. rapa* high oleic mutants suggest that a mutation causing a single amino acid change in *FAD2* can produce high oleic lines (Tanhuanpää et al. 1998) and thus a single functional copy of *FAD2* is responsible for seed oil composition. Furthermore, the introduction of a *B. rapa* *FAD2* antisense cDNA sequence led to high oleic transgenic *B. juncea* lines via gene silencing (Sivaraman et al. 2004). In *B. napus*, oleic acid content has been increased as high as 85% with an ihpRNA *FAD2* and *FAE* construct (Peng et al. 2010).

The oil profile of conventional *B. carinata* is characteristically high in erucic acid (22:1 c13; 40%) with approximately 2.6% stearic (18:0), 10% oleic, 18% linoleic and 16% linolenic acid. Furthermore, all wild-type strains or gene bank accessions examined to date are high in glucosinolate content, with the primary glucosinolate (>95%) being 2-propenyl. Clearly the wildtype profile is not particularly useful in food or meal applications. Initial breeding efforts at AAFC Saskatoon, Canada focused on *B. carinata* as a potential food crop leading to the development of low erucic material (Getinet et al. 1994); however, more recent efforts have focused on the production of unique fatty acid profiles for the industrial market with high erucic acid being a primary target.

Increases in erucic acid content are crucial for oil destined for the industrial market (Scarth & Tang, 2006). Transgenic *B. carinata* has been made which contains both the *Crambe abyssinica* *FAE* gene and an ihpRNA *FAD2* construct. This resulted in substantial increases in erucic acid levels, reaching as high as 56% (Mietkiewska et al. 2008). A transgenic approach has also been utilized in *B. napus*. The *LPAAT* gene from *Limnanthes douglasii* was successfully inserted, along with over expression of *Bn-fae1*, which has resulted in erucic acid levels of 72% (Nath et al. 2009). In addition to oil products, the development of markets for secondary products is crucial. The glucosinolate profile of *B. carinata* allows for its use as a bio-pesticide (Rongai et al. 2008). Furthermore, non-transgenic meal could be utilized as a feed stock for fish (Taylor et al. 2010).

EXPERIMENTAL

Interspecific crosses between the *B. rapa* breeding line CZY and the *B. juncea* line 60143 were made by Love et al. (1990) and following subsequent backcrosses to the zero erucic *B. juncea* line LDZ, both high oleic and moderate oleic lines were identified. Material investigated in the current study was developed from a cross between the high oleic *B. juncea* cultivar Arid (67.0% 18:1) and the moderate oleic acid breeding line 7285-8 (50.9% 18:1). Microspore derived doubled haploid (DH) material was grown in the field in 2003. Seed from each DH was profiled for fatty acid composition by gas chromatography, according to the method of Thies (1974).

The mean oleic acid content in this population was 46.15%. From the distribution it is apparent that within canola-quality *B. juncea* OAC is a multi-genic trait. Yet, from the *FAD2B* analysis, it was possible to divide the population into two distinct qualitative groups. The *FAD2B* locus was present in all DH lines that contain low to moderate OAC (<55%) whereas it was absent in lines with high oleic acid (>55%). A framework map containing 467 loci was created utilizing the DH population. This was further aligned with a much larger AAFC SSR *B. juncea* map (unpublished). The AAFC SSR map revealed that the first 20 cM of chromosome J11 may have been lost in the high oleic *B. juncea* lines.

Interspecific crosses were also carried out between the canola-quality *B. juncea* breeding line VR07-358 and the *B. carinata* breeding lines 080793EM (high C22:1) and 58EM-B10 (low C22:1) in order to transfer the *FAD2B* mutation. Each backcross generation was self pollinated and screened with the *FAD2B* marker. Fifteen individual plants from two high erucic BC₄F₃ (VR10-183 & VR10-192) lines and two low erucic BC₄F₄ lines (VR10-662 & VR10-680) were grown in the greenhouse and fames were determined using gas chromatography (Thies, 1974). Compared to the high erucic parent, oleic acid levels increased ~5% with a corresponding decrease in both C18:2 and C18:3 levels (Table 1); erucic acid levels also increased by ~4%. In the low erucic lines, oleic acid content was increased by ~14% with a corresponding decrease in C18:2 levels.

line	c181	c182	c183	c200	c201	c220	c221	c222	c223	c240	c241
080793EM	6.393	15.499	13.368	0.566	5.476	0.637	47.575	1.659	0.524	0.536	2.210
VR10-183	11.018	10.886	10.932	0.502	6.108	0.466	51.789	0.815	0.305	0.358	2.120
VR10-192	12.552	9.952	10.850	0.456	6.590	0.386	51.140	0.693	0.276	0.313	2.174
58EM-B10	29.289	35.028	26.134	0.479	1.102	0.245	0.055	0.000	0.000	0.214	0.491
VR10-662A	44.583	23.355	21.030	0.680	1.687	0.385	0.074	0.000	0.000	0.420	0.623
VR10-680A	41.931	24.339	21.920	0.610	1.520	0.380	0.474	0.000	0.000	0.291	0.668

Table 1: Fatty Acid profiles of *B. carinata* material

CONCLUSIONS

The oleic and erucic acid profiles of *B. juncea* and *B. carinata* were successfully altered through interspecific crosses. The original interspecific cross with *B. rapa* most likely resulted in a deletion event which removed a portion of chromosome J11 that contained the *FAD2B* allele. Such results are not unexpected as previous analysis of early material from the original *B. rapa* / *B. juncea* cross identified a low glucosinolate nullisomic line (Cheng et al. 2001).

The loss of the *FAD2B* allele increases OAC to levels greater than 55% in *B. juncea*. It is interesting to note that the oleic acid content in *B. carinata* only reached 44%.

It appears that in canola quality *B. juncea*, one gene, or a tightly linked cluster of genes, is primarily responsible for OAC in the developing seed. The loss of this allele greatly altered the fatty acid profile even though other copies of *FAD2* were present. Clearly other genes are involved in OAC in canola quality *B. juncea*. For example, we observed a range (up to 65%) in OAC among lines that contain the *FAD2B* deletion. Such observations are not surprising since previous *B. napus* analysis has located minor QTLs dispersed throughout the genome (Hu et al. 2006). These minor genes will become important once the major genes are fixed. Work in other Brassica species, including *B. napus* and *B. rapa*, also suggest a single functional copy of *FAD2* was present and responsible for the majority of the variation observed in OAC in developing seeds. The current low erucic acid *B. carinata* observations suggest that the *FAD2C* gene is more important in this species since the OAC content increased only to moderate levels with both *FAD2B* and *FAE* mutations.

In high erucic *B. carinata* it was possible to further increase erucic acid levels with the introduction of the *FAD2B* mutation. Substantial amounts of oleic acid are still being converted to linoleic and linolenic acid in this material, suggesting that additional improvements could be made. These observations are in agreement with those noted in transgenic *B. carinata* lines containing the ihpRNA *FAD2* construct. The levels of both linoleic and linolenic acid were substantially reduced as the construct would silence all copies of *FAD2* (Mietkiewska et al. 2008). This is further supported by the low erucic *B. carinata* data again suggesting the *FAD2C* gene as a likely target for future work.

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