Broadening genetic diversity in canola: development of double-low recombinant inbred lines from *Brassica napus* x *B. oleracea* cross

Rick A. Bennett¹, Mohan R. Thiagarajah¹, Ginette Séguin-Swartz², Habibur Rahman¹.

¹University of Alberta, Agricultural Food and Nutritional Science, Edmonton AB, Canada
²Agriculture and Agri-Food Canada, Saskatoon Research Centre, Saskatoon SK, Canada

Email: habibur.rahman@ualberta.ca

Abstract: The objective of this research was to broaden genetic diversity in *Brassica napus* (2n=38, AC genome) through exploitation of the C-genome of *B. oleracea* (2n=18, C genome). Sixty-five F₈ recombinant inbred lines (RILs) were developed from a wide cross between *B. napus* cv. Hi-Q (zero-erucic, 10 to 12 µmol glucosinolate/g seed) and *B. oleracea* var. alboglabra (40% erucic acid, >80 µmol glucosinolate/g seed). Half-seed fatty acid analysis, near-infrared spectroscopy (NIRS), flow cytometry, and molecular markers were used to develop double-low (=canola quality; zero-erucic, <30 µmol glucosinolate/g seed) *B. napus* type plants and to assess diversity among these lines. Seeds from 72% of the zero-erucic F₆ families had low (<30 µmol g seed⁻¹) glucosinolate content. Flow cytometry analysis of F₈ plants showed no significant difference from the *B. napus* cv. Hi-Q parent for nuclear DNA content. Using simple sequence repeat (SSR, microsatellite) markers, F₈ lines with 0.0% to 72.7% alleles originating from the *B. oleracea* parent were identified. This study demonstrates that it is feasible to broaden genetic diversity in double-low, *B. napus* type lines using the *B. oleracea* C-genome.

Keywords: *Brassica napus*, *Brassica oleracea* var. alboglabra, genetic diversity, erucic acid inheritance, flow cytometry, SSR markers

Introduction

The narrow genetic base of *B. napus* has been of concern to many researchers, as diversity is critical for continued improvement of the crop, as well as for adaptation to changing environments and markets (Cowling 2007). Using SSR markers, Hasan et al. (2006) found that among 96 rapeseed accessions from European genebanks tested, spring oilseed types had the lowest number of unique alleles (not present in other groups) compared to the number of unique alleles in winter and vegetable types. A loss of genetic diversity among contemporary Australian spring type cultivars (Cowling 2007) and low levels of genetic diversity among spring rapeseed accessions compared to Chinese semi-winter and interspecific-derived types (Qian et al. 2006) have also been reported. Thus, there is a need for broadening genetic diversity in spring type *B. napus* cultivars, which are most commonly grown in Northern Europe, Canada, and Australia.

U (1935) suggested the amphidiploid nature of *B. napus*, which arose from diploid progenitor species *B. rapa* (2n = 20, A genome) and *B. oleracea*, and this has been reconfirmed by Parkin et al. (1995) based on genetic linkage mapping. Within each diploid species there is a vast amount of morphological diversity, and the diploid genomes are genetically distinct from the corresponding genomes in *B. napus* (Song et al. 1988). Therefore, these diploid species are viewed as an important reservoir for increasing genetic diversity in *B. napus*. Two major constraints of utilizing the genetic diversity of *B. oleracea* for the improvement of *B. napus* are the difficulty of producing hybrids (Bennett et al. 2008), as well as obtaining canola quality euploid *B. napus* (2n=38) plants, from interspecific hybridization between these two species. Up to date, not much effort has been made on utilizing the C-genome for increasing genetic diversity in *B. napus*.

Our objective is to broaden genetic diversity in canola *B. napus* through exploitation of the C-genome of *B. oleracea*. Seeds of *B. oleracea* carry non-canola quality traits, such as high contents of erucic acid (~40%) in oil and glucosinolates (>80 µmol g seed⁻¹) in meal. Thus, the purpose of this research was to extend our understanding on the inheritance of erucic acid in the F₂ population of a *B. napus* x *B. oleracea* cross; as well as to investigate response to selection for low glucosinolate content and *B. napus* plant in different generations in the development of canola quality interspecific euploid *B. napus* RILs. For this purpose, we used *B. oleracea* var. alboglabra as model due to its spring growth habit and self-compatibility nature.

Materials and methods
Two zero-erucic, low glucosinolate (10 to 12 μmol g seed$^{-1}$) *B. napus* L. doubled haploid lines ‘Hi-Q’ and ‘A01-104NA’ were pollinated with a high erucic (40% erucic acid), high glucosinolate (>80 μmol g seed$^{-1}$) self-compatible inbred (F$_7$) *Brassica oleracea* var. *alboglabra* Bailey line and in vitro ovule culture technique was applied for generation of interspecific F$_1$ hybrid plants. Twelve (A01-104NA x *B. oleracea*) and five (Hi-Q x *B. oleracea*) F$_1$ plants were manually self-pollinated for generation of F$_2$ seed. A total of 93 F$_2$ seeds, 52 from A01-104NA x *B. oleracea* and 41 from Hi-Q x *B. oleracea*, were analyzed for fatty acid profile by half-seed fatty acid analysis technique. The details of this technique are described elsewhere (Bennett et al. 2008). F$_3$ to F$_8$ generation populations were grown either in greenhouse (in winter) or in field plots (in summer) at the Edmonton Research Station of the University of Alberta. A total of 65 F$_8$ Hi-Q x *B. oleracea* RILs were developed through pedigree selection.

Bulked seed harvested from F$_4$ and F$_6$ generations grown in replicated field plots were analyzed for glucosinolate content using NIRS (FOSS NIRSystems model 6500). The details of this analysis are described elsewhere (Kebede et al. 2010). The glucosinolate content for each seed family was determined using the average value of two replications. Ploidy in the 65 F$_8$ RILs was estimated by flow cytometry. For this, leaf tissue (~1 cm$^2$) from seedlings at the age of 3 to 5 weeks after seeding were excised and chopped with a razor blade in Partec buffer supplemented with nuclear fluorochrome DAPI (4,6-diamidino-2-phenylindole, Sigma, product no. D-9542). Samples were filtered using Partec CellTrics$^{(TM)}$ fitted with nylon gauze (30 μm pore size) and run through a Partec Ploidy Analyzer (Partec GmbH, Münster, Germany). These 65 F$_8$ RILs were also subjected to SSR marker analysis to obtain genotypic data. DNA extraction, polymerase chain reactions (PCR) and labeling of PCR products were done as described by Kebede et al. (2010). The two parents (Hi-Q and *B. oleracea*) were screened for polymorphic marker alleles using 191 SSR primer pairs. Based on distinct bands and clear polymorphism, a total of 26 SSR markers from the 9 C-genome linkage groups and 3 unmapped markers, were used for genotyping the RILs.

**Results**

Seeds of the two *B. napus* parents, Hi-Q and A01-104NA, contained 0.4% erucic acid; while seeds of *B. oleracea* contained approximately 40% of this fatty acid. The distribution of the F$_2$ seeds for erucic acid content is presented in Fig. 1. The average erucic acid content of all F$_2$ seeds was 15.4%, which was significantly lower than the mean of the two parental species (t = -4.76, P<0.01). A clear zero-erucic acid class was observed, which included nine seeds. Erucic acid content in the remaining 84 seeds ranged between 4 and 43%. Four rough peaks were observed among seeds containing erucic acid: the highest numbers occurred at 8 to 10% and again at 18% erucic acid content; while a small number fell at 33% and >40% content of this fatty acid (Fig. 1). The 93 F$_2$ seeds produced 72 mature F$_2$ plants. Of these, twenty were sterile; while the remaining 52 plants were at least partially fertile. Good plant fertility was correlated with lower levels of erucic acid content. The average erucic acid content of the F$_2$ seeds resulting in these fertile or semi-fertile F$_2$ plants was 11.2% (range 0 to 24%).

![Fig. 1: Distribution of the F$_2$ seeds of *B. napus* x *B. oleracea* for erucic acid content (n = 93). Seeds fall into five rough groups based on phenotype: I) zero-erucic acid class, II) low erucic acid class, III) intermediate erucic acid class, IV) moderate-high erucic acid class, and V) high erucic acid class. Percentages along the top of each class indicate proportion of total plants that produced F$_3$ seeds.](image-url)
Glucosinolate content of Hi-Q, grown together with different segregating populations ranged between 6.7 and 11.1 μmol g seed⁻¹, with a mean of 9.8 ± 1.5. In the F₄ generation, glucosinolate content among the 20 plant families ranged from 14.6 to 67.5 μmol g seed⁻¹ with a mean of 37.8 ± 13.6. For this population, 7 families (35%) had low (<30 μmol g seed⁻¹) glucosinolate content and 1 (5%) family had a content similar (<15 μmol g seed⁻¹) to the B. napus parent. The 25 F₆ families had a glucosinolate content ranging from 10.6 to 50.7 μmol g seed⁻¹, with a mean of 24.7 ± 10.7. For this population, 18 families (72%) had low glucosinolate content, and 7 (28%) were similar to the B. napus parent.

The B. napus parent Hi-Q had a Partec value of 195.7; while the B. oleracea parent had a value of 117.4. The RILs derived from these two parents had a range of 175.0 to 208.0 and averaged 196.3 ± 4.9 (Table 1). No significant difference was found between the mean of this population and the Hi-Q parent (t=1.02, P=0.31); however, it was significantly different than the B. oleracea parent (P<0.01).

Table 1: Nuclear DNA content, estimated by flow cytometry analysis, in B. napus and B. oleracea var. alboglabra parents and their F₈ inbred lines.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>No. plants</th>
<th>Mean Partec value</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. napus ‘Hi-Q’</td>
<td>2</td>
<td>195.7</td>
<td>193.0 – 198.4</td>
</tr>
<tr>
<td>F₈ RILs</td>
<td>64</td>
<td>196.3 ± 4.9</td>
<td>175.0 – 208.0</td>
</tr>
<tr>
<td>B. oleracea</td>
<td>2</td>
<td>117.4</td>
<td>116.5 – 118.3</td>
</tr>
</tbody>
</table>

Molecular marker analysis revealed the occurrence of B. oleracea alleles among 64 RILs ranged from 0.0 to 72.7% with a mean value of 23.6%. The F₈ lines (n=52) originating from F₆ families with double-low seed quality (zero-erucic, ≤30 μmol g seed⁻¹) had an average of 20.2% (range 0.0 to 72.7%) alleles from B. oleracea; while the 12 non-canola quality type lines (>30 μmol g seed⁻¹) averaged 38.1% (range 11.8 to 64.7%).

Discussion

Introgression of genetic diversity into B. napus from its parental species B. oleracea imposes a greater challenge compared to introgression of genetic diversity from B. rapa, which is primarily due to high glucosinolate and high erucic acid in this donor species. In this study, zero-erucic acid and low glucosinolate content (double-low) genotypes were obtained already in the F₄ generation from a relatively small segregating population. Among the 52 double-low B. napus type RILs, an average of 20% marker alleles originated from the B. oleracea parent. These genetically diverse lines will be used in the breeding program for enhancement of specific traits and may have great potential as a heterotic pool in hybrid canola breeding.

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References

UN (1935) Jpn J Bot 7:389-452