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

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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 F_8 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 F_6 families had low (≤30 µmol g $seed^{-1}$) glucosinolate content. Flow cytometry analysis of F_8 plants showed no significant difference from the *B. napus*cv. Hi-Q parent for nuclear DNA content. Using simple sequence repeat (SSR, microsatellite) markers, F_8 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 accessionscompared 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 F_2 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 appliedfor generation of interspecific F_1 hybrid plants. Twelve (A01-104NA x *B. oleracea*) and five (Hi-Q x *B. oleracea*) F_1 plantsweremanually 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 plotswere 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-diaminido-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%. Fourrough peaks were observed among seeds containing erucic acid: the highest numbers occurredat 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 theremaining 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 resultingin these fertile or semi-fertile F_2 plants was 11.2% (range 0 to 24%).

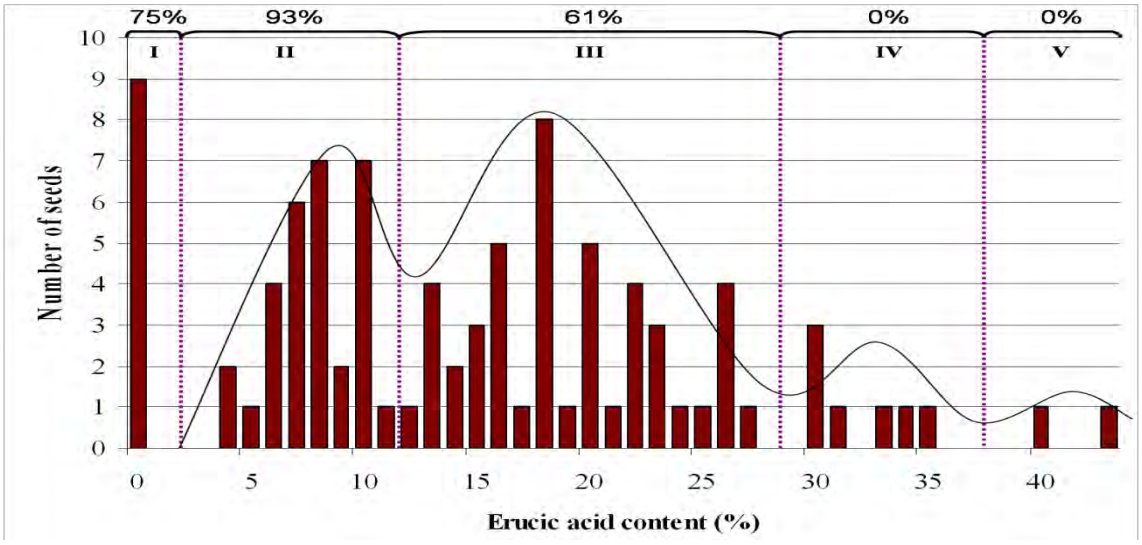

Fig. 1: Distribution of the F_2 seeds of *B. napus* x *B. oleracea* for erucic acid content (n = 93). Seeds fall into five rough groups based on phenotype: I) zero-erucic acid class, II) low erucic acid class, III) intermediate erucic acid class, IV) moderate-high erucic acid class, and V) high erucic acid class. Percentages along the top of each class indicate proportion of total plants that produced F_3 seeds.

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 F_4 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 F_6 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*parentHi-Qhad 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 F_8 inbred lines.

Genotypes	No. plants	Mean Partec value	Range
B. napus 'Hi-Q'	2	195.7	193.0 – 198.4
F_8 RILs	64	196.3 ± 4.9	175.0 – 208.0
B. oleracea	2	117.4	116.5 – 118.3

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 F_8 lines (n=52) originating from F_6 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 F_4 generation from a relatively small segregating population. Amongthe 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

Bennett RA, Thiagarajah MR, King JR, Rahman MH (2008) Euphytica **164**:593-601
Cowling WA(2007) Field Crop Res**104**:103-111
Hasan M, Seyis F, Badani AG, Pons-Kühnemann J, Friedt W, Lühs W, Snowdon RJ (2006) Genet Resour Crop Evol**53**:793-802
Kebede B, Thiagarajah MR, Zimmerli C, Rahman MH (2010) Crop Sci**50**:1236-1243
Parkin IAP, Sharpe AG, Keith DJ, Lydiate DJ(1995) Genome **38**:1122-1131
Qian W, Meng J, Li M, Frauen M, Sass O, Noack J, Jung C (2006) Theor Appl Genet **113**:49-54
Song KM, Osborn TC, Williams PH (1988) Theor Appl Genet**75**:784-794
U N (1935) Jpn J Bot **7**:389-452