

Developing Genetic Resources For *Brassica carinata*

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Introduction

Brassica carinata (2n = 34, BBCC) (Ethiopian Mustard, Abyssinian mustard) has desirable qualities, including disease resistance (black leg, alternaria leaf spot), insect resistance (flea beetles, aphids) and drought tolerance, suitable for a new Prairie crop platform (Fig. 1 and 2). The crop could provide new sources of sustainable non-food raw material either biomass to be processed into energy or oil which could be transformed into liquid biofuels or erucic acid derivatives for the plastic industry. Traditional breeding efforts are underway to improve such traits as oil content, yield, crop architecture and introduce early flowering alleles. Since there are currently limited genetic resources available for molecular breeding in *B. carinata*, we are developing a robust set of genome-wide markers for rapid trait introgression. Thirty-nine *B. carinata* lines selected from collections around the world were screened using a set of A/C (*B. napus*) and B (*B. juncea* and *B. nigra*) genome Brassica simple sequence repeat (SSR) markers to determine the genetic diversity represented among the lines. Six of the most genetically distinct lines are being used for deep 3' based transcriptome sequencing (Roche 454) to identify single nucleotide polymorphisms (SNPs). Initial sequencing efforts have focused on the parental lines from a segregating doubled haploid population to allow the SNP loci to be placed on a genetic linkage map. The developed SNP markers and associated genetic map will be a powerful tool for marker-assisted breeding in *B. carinata*.

Objectives

The objective of this work was to screen selected *B. carinata* lines using a set of A/C (*B. napus*) and B (*B. juncea* and *B. nigra*) Brassica genome simple sequence repeat (SSR) markers. The SSRs are being used to determine the level of genetic diversity and distinctiveness between the lines. Two of the selected lines are the parents of a doubled haploid population (developed at the John Innes Centre) which will be used to generate a genetic reference map for *B. carinata*. The use of a common set of markers will allow this map to be aligned with linkage maps from *B. napus* and *B. juncea*.



Figure 1. Eight week old *B. carinata* plant grown in the greenhouse.



Figure 2. Field grown *B. carinata*, showing variation for flower colour. Photo donated by Kevin Falk, AAFC, Saskatoon Research Centre

Simple Sequence Repeat Screening

- Thirty-nine *B. carinata* lines collected all over the world were screened using 120 SSR markers established from:
 - B. napus* (A and C genome)
 - B. juncea* and *B. nigra* (B genome)
- DNA from the 39 *B. carinata* lines were amplified through PCR using a M13-21 tailed fluorescently-labelled primer
- Amplified SSR fragments were analysed using an Applied Biosystems 3730XL DNA Analyzer
- Data Analysis was performed using the program Genographer
 - Measures the fragment size of base pairs (bps) amplified from the SSR markers

Results and Discussion

Fragment sizes (alleles) generated from each SSR marker were compared between each *B. carinata* line (Fig. 3). Twenty six SSR markers did not produce bands. The majority of the lines selected from the genetic resource centre appeared homozygous with a select few expressing both parental alleles (heterozygous) at a subset of the queried loci.

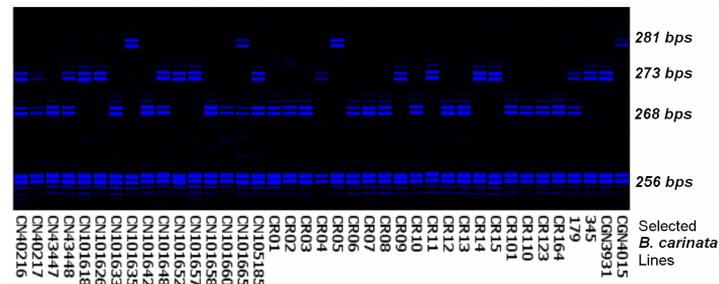


Figure 3. SSR marker sA1315 on selected *B. carinata* Lines

The fragment sizes were converted to binary format and scoring was determined by the presence of a band "1" and the absence of a band "0". The abbreviation N/A was given if there were no bands present in the lane and was not included in the calculation. The average dissimilarity scores (ADS) was calculated to measure the overall genetic difference present between the lines of interest. A higher ADS obtained between unlinked SSR markers means that the specific line has a distinct genetic background compared to other lines. The results presented in Table 1 followed the procedures and calculations as described by Fu (Fu, 2006).

Table 1. Calculated mean of the dissimilarity scores determined from pairwise comparisons across all lines.

Line	ADS	St. Dev.	Line	ADS	St. Dev.	Line	ADS	St. Dev.
CN40216	0.129	0.023	CN101660	0.097	0.027	CR11	0.111	0.030
CN40217	0.107	0.020	CN101665	0.124	0.035	CR12	0.092	0.026
CN43447	0.112	0.022	CN105185	0.128	0.021	CR13	0.092	0.026
CN43448	0.145	0.026	CR01	0.109	0.030	CR14	0.108	0.030
CN101618	0.115	0.015	CR02	0.113	0.029	CR15	0.126	0.030
CN101626	0.145	0.021	CR03	0.121	0.027	CR101	0.108	0.034
CN101633	0.129	0.025	CR04	0.121	0.030	CR110	0.097	0.033
CN101635	0.132	0.019	CR05	0.106	0.027	CR123	0.142	0.020
CN101642	0.123	0.024	CR06	0.097	0.027	CR164	0.102	0.027
CN101648	0.127	0.024	CR07	0.139	0.024	179	0.125	0.026
CN101652	0.135	0.022	CR08	0.118	0.025	345	0.129	0.029
CN101657	0.113	0.026	CR09	0.105	0.027	CGN3931	0.118	0.027
CN101658	0.119	0.027	CR10	0.108	0.029	CGN4015	0.129	0.024

Conclusion and Future Work

From the SSR work, lines CN43448, CR123, CN101626, CR07, CN101652, and CN101635 were determined to have the highest ADS showing a genetic background more distinct than the rest of the selected lines. These lines will be looked at more closely along with the parents of the mapping population (179 and 345).

The most diverse *B. carinata* lines will be used to generate a set of polymorphic SNPs markers for in-depth genetic mapping and genome analysis in *B. carinata*. SNP variation will be identified through next generation sequencing of 3' biased cDNA libraries, which has proved very successful in *B. napus*. With future crop work relying on marker assisted breeding, creating a robust set of genetic markers and an associated genetic linkage map will be very valuable for future molecular research in *B. carinata*.

References

Fu, Y.B. 2006. Genetic redundancy and distinctiveness of flax germplasm as revealed by RAPD dissimilarity. *Plant Genet. Resour.* 4:117-124.

Acknowledgements

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