Introgression of blackleg (*Leptosphaeria maculans*) resistance into *Brassica napus* from *B. carinata* and identification of microsatellite (SSR) markers

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Abstract

An interspecific cross between a blackleg susceptible *B. napus* cv. Westar and a resistant accession of *B. carinata* was accomplished, and the *B. napus*×*B. carinata* F_1 hybrids were backcrossed to Westar two times and self-pollinated three times and several BC_2S_3 families were generated. Four BC_2S_3 families of canola quality were used for doubled haploid (DH) production. Doubled haploid populations derived from the two BC_2S_3 families were found to carry cotyledon as well as adult-plant resistance to *Leptosphaeria maculans* pathotype PG2 isolate #UA3356. Microsatellite markers linked to the *B. carinata*-derived resistance genes in *B. napus* were identified. These resistant lines coupled with the identified molecular markers could be useful in breeding and research programs for broadening the resistance gene pool in *B. napus*, and for pyramiding the disease resistance genes for quantitative resistance in future *B. napus* cultivars to improve durability of resistance to blackleg.

Key words: Canola – Brassica napus – blackleg – interspecific gene transfer – molecular marker – SSR

Introduction

Canola (*Brassica napus*) is a $3 billion crop in Canada – In the last few years, resistance to diseases such as blackleg stem canker (causal agent, *Leptosphaeria maculans* / *Phoma lingam*) and *sclerotinia* stem rot (causal agent, *Sclerotinia sclerotiorum*) have been much desired by Canadian canola growers as these diseases cause significant yield loss. Yield loss due to blackleg alone can be up to 20% (Schramm and Hoffmann 1992).

Apart from the sources of blackleg resistance available in existing *B. napus* germplasm, excellent resistance has also been reported in several accessions of *Brassica* that carry the B-genome (Sjödin and Glimelius 1988). *Brassica carinata* carries unique resistance to blackleg, which apparently is superior to other sources of resistance available in *B. napus* as it can be screened at the cotyledon stage (Bansal et al. 2000). Furthermore, this species carries excellent resistance to different blackleg pathotypes including PG3, which is becoming an increasing concern to the canola growers in Canada. The objective of the research project was to introgress blackleg resistance from *B. carinata* into canola *B. napus* through interspecific hybridization, and to identify molecular marker(s) linked to the resistance gene(s). Introgression of blackleg resistance from *B. carinata* into *B. napus* is expected to improve blackleg resistance in this crop; as well as to result in greater diversity for the resistance genes.

Materials and methods

A blackleg resistant *B. carinata* accession #98-14513 and a blackleg susceptible *B. napus* cultivar Westar were used. Crosses between Westar and *B. carinata* was done using Westar as female. The F_1 hybrids were backcrossed with Westar (male) two times and self-pollinated three times with repeated selection for blackleg resistance (Bansal et al. 2000) and several BC_2S_3 families were generated. Four BC_2S_3 families of canola quality (zero erucic and low glucosinolate) were used for doubled haploid (DH) production.

The DH lines were evaluated for resistance to blackleg pathotype PG2 (#UA3356, Canola Breeding program of the University of Alberta) at cotyledon and adult plant stage. Classification for resistance was done on a qualitative scale: Resistant (R), where lesion size was similar to *B. carinata* parent or susceptible (S), where lesion size was similar to Westar or moderately resistant (MR), where lesion size was intermediate between these two classes.

Microsatellites markers (SSR) were applied for identification of markers linked to the resistance gene(s). The SSR markers and the linkage map of *B. napus* developed at the AAFC Saskatoon Research Centre (http://www.intl-pag.org/11/abstracts/P5e_P473_XI.html) were used for this purpose. In the first step, bulk segregant analysis was performed on the resistant (*B. carinata*) and susceptible (Westar) parents and bulk sample of resistant and susceptible DH populations. In case of DH population, each bulk consisted of 15-18 DH lines that were either resistant or susceptible. The rationale of bulk segregant analysis was to identify polymorphisms simultaneously in both the parental lines and in samples of resistant and susceptible DH lines. Once potential association of marker and gene is established, in the second step, fine scale

mapping was done using the individual DH lines.

Genomic DNA from young leaves (8-10 weeks) of the parents and DH lines was extracted using SIGMA GenElute™ Plant Genomic DNA Miniprep Kit. The Molecular Probes™ Quant-It™ PicoGreen® dsDNA Assay Kit was used to quantify the DNA concentration of the extracted tissue and 20ng of DNA was used for the amplification reactions. Each PCR reaction consisted of the following components to a final volume of 10µl: ddH_20 2.2 µL, 10X PCR buffer 1.0 µL, 25X dNTP's 1.0 µL, $MgCl_2$ 1.0 µL, forward primer 1.25 µL, reverse primer 1.25 µL, M13 label 0.1 µL, Taq polymerase 0.2 µL, Template DNA 2.0 µL. Both forward and reverse primers were synthesized by Alpha DNA. A Licor M13 labeled sequence "5'-CACGACGTTGTAAAACGAC-3" was added to the forward primer. The *Taq* DNA polymerase was obtained from 'Invitrogen Platinum'.

The following PCR protocol was run on a MJ Research P-100 thermocycler: 95°C for 5 minutes (incubation) → 95°C for 15 seconds (melt) → 57°C for 15 seconds (anneal) → 72°C for 1 minute (extension) → step 2 to 4 (melt – extension) for 35 cycles → 72°C for 7 minutes (final extension) → 4°C (hold). Samples were run on a 7% polyacrylamide gel at 1500 volts and 50°C for 3 hours, and the amplified fragments were visualized using a Licor 4200 Global IR^2 System.

Results

Of the four DH populations derived from four BC_2S_3 families, cotyledon resistance was found in the DH lines of two populations: Popl#3 (02-17006) and Popl#4 (02-17009). The DH Popl#3 segregated for blackleg resistance in 1:1 ratio (33 resistant/moderately resistant: 34 susceptible, $\chi^2 = 0.015$, p = 0.90 – 0.95) and was used for SSR marker identification. On the other hand, almost all DH lines of the Popl#4 were resistant to blackleg. One resistant DH line from this population was crossed with Westar and 32 DH lines were produced from the F_1 which is referred as Popl#4.1. In this population also, a 1:1 segregation (14 resistant/moderately resistant: 18 susceptible, $\chi^2 = 0.50$, p = 0.3 – 0.5) for blackleg resistance was found, and was used for SSR marker identification. A total of 256 SSR primers covering 412 loci were tested on the *B. carinata* and *B. napus* (Westar) parents, where ca. 60% of the tested markers showed polymorphism.

As most of the DH lines derived from BC_2S_3 family 02-17009 (Popl #4) were resistant to blackleg at cotyledon stage, randomly selected 27 DH lines from this population together with the parents' *B. carinata* and Westar were evaluated using the polymorphic SSR markers. Of the tested markers, sN9756 (Primer #46) co-segregated with the resistant DH lines as well as was present in *B. carinata* but not in Westar. This primer pair amplified six fragments of approximately 430 – 480 bp, of which the fragment of 430 bp size showed correlation with blackleg resistance at cotyledon stage.

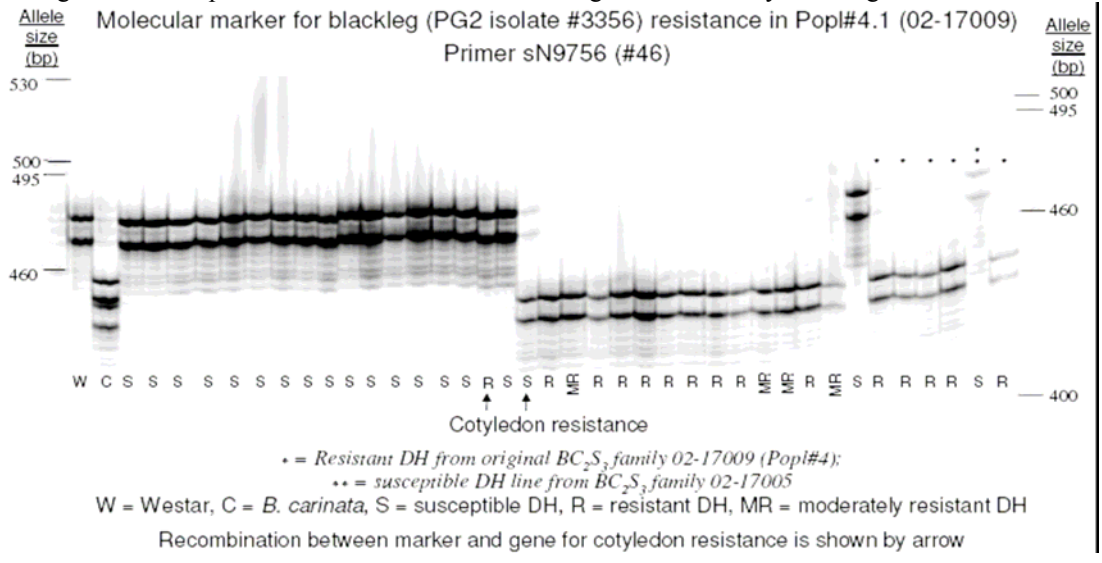

Fig. 1. Microsatellite (SSR) marker sN9756 linked to blackleg resistance to pathotype PG2 (isolate #UA3356) in Popl#4.1 (02-17009) that introgressed into LG15 of *B. napus* from *B. carinata*.

Fourteen resistant and 18 susceptible DH lines of the Popl#4.1, i.e. derived from the F_1 of resistant DH of Popl#4 crossed with Westar, were evaluated. In this population, the same marker sN9756 (Primer #46) also co-segregated with cotyledon resistance (Fig. 1) except two cases, and this translated to 6.3% recombination between the marker and the resistance gene. Adult plant resistance fairly correlated with the cotyledon resistance. Of the 14 cotyledon resistant DH lines, 13 were tested for adult plant resistance and all showed good resistance (resistant or moderately resistant) at this stage as well. In contrast, of the 18 cotyledon susceptible DH lines, four were moderately resistant and one resistant at adult plant stage. Apart from some discrepancy between cotyledon and adult plant resistance, selection by use of the identified SSR marker can effectively discard most of the susceptible genotypes. Based on the *B. napus* SSR linkage map, it is anticipated that the marker sN9756 (Primer #46) is located on LG15, i.e. resistant gene has been introgressed from *B. carinata* into the LG15 of the C-genome of *B. napus*. In this linkage group, a total of 25 other markers were evaluated where none of these showed polymorphism with resistance.

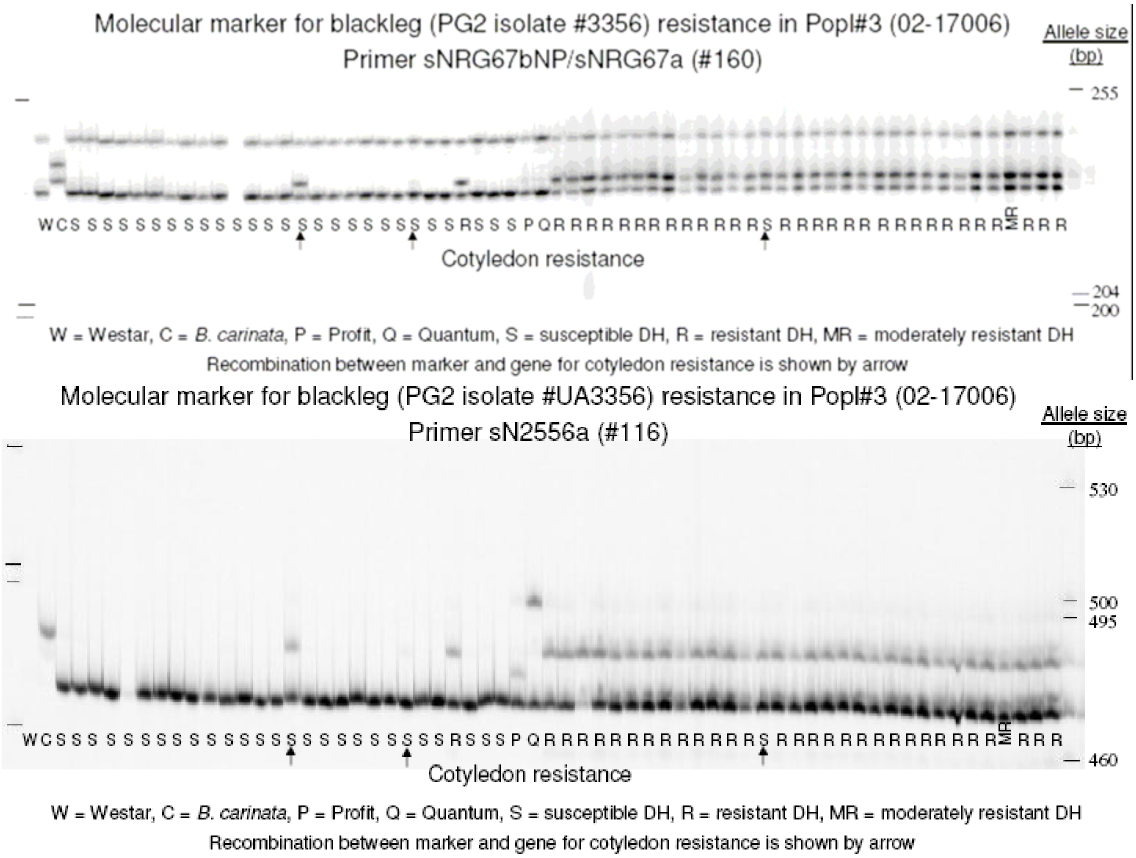

Fig. 2. Microsatellite (SSR) markers sNRG67bNP and sN2556 linked to blackleg resistance to pathotype PG2 (isolate #UA3356) in Popl#3 (02-17006) that are introgressed into LG3 of *B. napus* from *B. carinata*.

In the case of the DH lines derived from the Popl#3 (02-17006), randomly selected 60 lines, comprising of 32 resistant and 28 susceptible at cotyledon stage, were used for identification of SSR markers. Six markers (sNRG67, sN4034, sN3850, sN8615, sNRG13, sN2556) co-segregated with cotyledon resistance. Recombination between the markers and the gene was estimated to be only 4.8%. Adult plant resistance correlated well with the cotyledon resistance, where more than 95% of the DH lines agreed with cotyledon test for resistance. The markers, sNRG67 and sN4034 amplify the genomic regions of LG3 as well as LG13; while the markers sN3850, sN8615 and sNRG13 amplify only the genomic region of LG3. A total of 39 loci covering the whole LG3 was evaluated of which these five loci (sNRG67, sN4034, sN3850, sN8615, sNRG13), which clustered together, showed linkage association with blackleg. On the other hand, 41 loci of LG13 were evaluated of which only the above-mentioned two loci (sNRG67 and sN4034) showed polymorphism. In case of this linkage group, two additional markers, which are located in the same position of sNRG67 and sN4034, and six markers which are located within a distance of 6.6 cM from these two markers, were tested but none of these showed linkage association with the resistant gene. As the markers sN3850, sN8615 and sNRG13 are not associated with the LG13, the occurrence of strong association between the amplified fragments of all these five markers and blackleg resistant gene suggest that the second resistant gene has been introgressed into LG3 of the A-genome of *B. napus*.

The marker sN2556, which also showed linkage association with blackleg resistance (Fig. 2), is actually located on LG10 in the *B. napus* SSR linkage map. However, this marker showed exactly the same linkage association as was found in case of the five markers on the LG3. It is most likely that the marker sN2556 is detecting a non-collinear locus in the introgressed segment (i.e. the marker locus detected by sN2556 is on a different chromosome relative to the other markers in *B.napus* while it is linked with the other markers in *B. carinata*). In LG10, 14 other markers were evaluated and none of these showed linkage association with blackleg resistance

Discussion

In the present study, blackleg resistance has been introgressed into *B. napus* from *B. carinata*. Molecular data suggest that at least two genes from *B. carinata* have been introgressed into *B. napus* – one being in the A- and one in the C-genome. These genes confer good resistance to blackleg isolate PG2 #UA3356 at cotyledon as well as at adult plant stage.

Introgression of more than one resistance gene into *B. napus* is possible if the donor parent *B. carinata* carries more than one resistance gene locus. Mapping of the *Brassica* B-genome indicated that this genome contains three complete, but rearranged copies of a fundamental ancestral genome (Lagercrantz 1998). This corroborates well with Zhu et al.'s (1993) finding that at least three chromosomes of *B. nigra* carry blackleg resistance. Similarly, Dixelius and Wahlberg (1999) suggested that the two amphidiploid species and the diploid species carrying the B-genome, *B. juncea*, *B. carinata* and

B. nigra, carry triplicated genomic regions for blackleg resistance, which reside in LG2, LG5 and LG8 of the B-genome.

Plieske et al. (1998) reported that one of the three B-genome resistance gene introgress in the same genomic regions of the *B. napus* genome independent of their origin (*B. nigra*, *B. juncea* and *B. carinata*) – apparently due to their preserved identity. This B-genome resistance gene seems to introgress into the A-genome (Plieske and Struss 2001). However, our study based on SSR markers, suggests that in addition to one genes introgressed into the A-genome one resistance gene had been introgressed into the C-genome.

Further study will be needed on the effect of combination of these resistance genes from A- and C-genomes on the resistance to different blackleg pathotypes as well as the further characterisation of the genetic organization of the introgressed segments.

Acknowledgments

We thank Alberta Agricultural Research Institute (AARI) and Alberta Crop Industry Development Fund (ACIDF) for financial support to this project and Ms. Julie Bernier for her help in disease screening.

References

Bansal VK, Stringam GR, Thiagarajah MR, Tewari JP, 2000. Introgression of multiple disease resistance traits from *Brassica carinata* into *B. napus*. Paper presented in international symposium: *Durable resistance, key to sustainable agriculture.* November 28 – December 1, 2000. Ede-Wageningen, The Netherlands.

Dixelius C, Wahlberg S, 1999. Resistance to *Leptosphaeria maculans* is conserved in a specific region of the *Brassica* B genome. Theor Appl Genet 99, 368-372.

Lagercrantz U, 1998. Comparative mapping between *Arabidopsis thaliana* and *Brassica nigra* indicates that Brassica genomes have evolved through extensive genome replication accompanied by chromosome fusions and frequent rearrangements. Genetics 150, 1217-1228.

Plieske J, Struss D, 2001a. STS markers linked to Phoma resistance genes of the *Brassica* B-genome revealed sequence homology between *Brassica nigra* and *Brassica napus*. Theor Appl Genet 102, 483-488.

Plieske J, Struss D, Robbelen G, 1998. Inheritance of resistance derived from the B- genome of *Brassica* against *Phoma lingam* in rapeseed and the development of molecular markers. Theor Appl Genet 97, 929-936.

Schramm H, Hoffmann GM, 1992. Fungicide effects on the development of *Phoma lingam* in winter oilseed rape. J Plant Dis Protection 99, 145-158.

Sjödin C, Glimelius K, 1988. Screening for resistance to blackleg Phoma lingam (Tode ex Fr.) Desm. within *Brassicaceae*. J Phytopathol 123, 322-332.

Zhu JS, Struss D, Robbelen G, 1993. Studies on resistance to *Phoma lingan* in *Brassica napus-Brassica nigra* addition lines. Plant Breed 111, 192-197.