Project: 2012F161R

Molecular cytogenetics of blackleg resistance in the *Brassica* B-genome, and introgression of resistance into *B. napus* through recurrent backcrossing

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Introduction

Blackleg disease in oilseed *B. napus* (AACC) causes yield loss up to 20% (Fitt et al. 2006; Gugel & Petrie 1992; Schramm & Hoffmann 1992). A number of partial resistance genes have been identified in *Brassica*, and most of these genes are located on the A7 chromosome of the A genome (for review, see Delourme et al. 2006). The Canadian canola breeders including the breeding program of the University of Alberta had been utilizing *Rlm4* (recent data indicate *Rlm3* which reside in the same genomic region of A7, personal communication with Dr. Nicholas Larken, AAFC), and *LmR1* resistances of the A7 linkage group of the Australian canola (*B. napus*) cultivars Maluka (Rimmer et al. 1999) and Shiralee (Mayerhofer et al. 1997) to develop blackleg resistant canola cultivars for the Prairie Provinces where PG2 pathotype has been most prevalent. However, the *Rlm4* resistance was found ineffective against the newly emerging pathotypes, e.g. PG3, PG4 and PGt, on the Canadian prairies (Fernando and Chen 2003; Chen & Fernando 2006; Kutcher et al. 2007). This raises concern to canola growers in Canada. Breakdown of single gene resistance has also been reported in Australia (Li & Cowling 2003; Sprague et al. 2006) and France (Brun et al. 2010). Therefore, it has become important to breed cultivars with different resistance genes for effective and durable resistance against the emerging *L. maculans* pathotypes.

Brassica species that contain the B-genome, namely *B. nigra* (BB, 2*n* = 16), *B. carinata* (BBCC, 2*n* = 34) and *B. juncea* (AABB, 2*n* = 36), are known to carry excellent resistance to blackleg that is effective throughout the life of the plant (Rimmer & van den Berg, 1992, Balesdent et al. 2001, Christianson et al. 2006). To date, several researchers carried out *B. napus* × *B. juncea* and *B. napus* × *B. nigra* interspecific hybridization for the introgression of B-genome resistance of *B. juncea* and *B. nigra* into *B. napus* (Roy 1978, 1984; Zhu et al. 1993; Chèvre et al. 1996, 1997; Saal et al. 2004). Success was reported with *B. juncea* where the introgressed gene showed resistance to a highly virulent isolate prevalent in Europe (Chèvre et al. 1997); however, it is not known whether the developed lines were resistant to *L. maculans* PG4-type isolates. On the other hand, only few researchers have attempted to transfer resistance from *B. carinata* into *B. napus* (Dixelius and Wahlberg 1999, Plieske et al. 1998), however, no report of success for commercial application can be found.

The focus of the canola breeding program at the University of Alberta in recent times has been to incorporate disease resistance genes from *B. carinata* into *B. napus* (Navabi et al. 2010). In the research project #2007F068R, funded by ACIDF/AFC/CC, which ended in March 2011, we conducted *B. napus* × *B. carinata* interspecific hybridization for introgression of PG4 type resistance from the B genome of *B. carinata* into the A/C genome of *B. napus*. In this project, we backcrossed the interspecific hybrids to *B. napus* with selection for cotyledon and adult plant resistance and developed several advanced generation self-pollinated families carrying five B genome chromosomes; where many of the families showed resistance to PG4 type blackleg pathotype. These materials were used in this research project for dissecting the B genome chromosome carrying resistance to PG4 type blackleg pathotype, as well as for introgression of PG4 resistance from *B. carinata* into *B. napus*.

Project objectives

With the above background, we submitted the research project 2012F161R to the Funding Consortium with the following objectives:

- identify the B genome chromosome(s) carrying the cotyledon and adult plant resistance through molecular cytogenetic study; and
- continue with recurrent backcrossing of the *B. napus* × *B. carinata* hybrids for introgression of *L. maculans* PG4 type resistance from the B genome of *B. carinata* into the A/C genome of *B. napus*.

Although project "2007F068R" officially ended in March, 2011, we continued with the research for about one and half year; and during this time we learnt more about the PG4-type resistance of *B. carinata*. In most cases, blackleg resistance in canola (*B. napus*) to relatively less virulent *L. maculans* isolates e.g. PG2, is under the control of a single gene locus. However, based on experience we gained with this interspecific cross (*B. napus* × *B. carinata*), it seems that resistance in *B. carinata* to more virulent PG4-type *L. maculans* isolates is under the control of more than one gene locus. This inference came from our observation that resistance often got lost in the segregating population – as it happens for traits controlled by the concerted effects of more than one gene. To achieve full resistance to *L.*

maculans PG4-type isolates in *B. napus*, introgression of all genes conferring PG4-type resistance in *B. carinata* may be needed.

Among the three *Brassica* genomes (A, B and C), the A and C genomes which are present in canola *B. napus*, are much closer to each other in terms of sequence homology than to the B genome. Hence the introgression of gene(s) from A to C or *vice versa* is relatively easy (Rahman 2001). On the other hand, the B genome, which is present in *B. carinata*, is very distant from the A and C genomes, and hence introgression of a gene from this genome into A or C genome of *B. napus* is a challenging task. Thus the introgression of multiple genes from the B to the A/C genome may be needed for introgression of PG4-type resistance from *B. carinata* into *B. napus*. This will be a much more daunting task – may need long time to accomplish (or even can be uncertain). As mentioned above, *B. carinata* show excellent resistance to *L. maculans* PG4 isolate; therefore, utilization of this germplasm for blackleg resistance in *B. napus* would be valuable in the long-term perspective. In this context, identification of the B-genome chromosomes of *B. carinata* carrying the resistance genes will be a step forward to achieving the goal. For this, generation of lines that carry single B-genome chromosome and multiple B-genome chromosomes would be needed.

Based on this background information, i.e. difficulty of introgression of PG4-type resistance from *B. carinata* into *B. napus* in the short-term, and the problems currently the canola industry faces with blackleg disease resistance, we in consultation with the Funding Consortium revised the research proposal for best use of our resources. In the revised version, major emphasis was given on the development of doubled haploid (DH) lines carrying different B genome chromosome(s), and identification of the B-genome chromosome or combination of chromosomes responsible for resistance *L. maculans* PG4-type isolate. Thus, the revised objectives of this project (2012F161R) were:

- To identify the B-genome chromosome(s) carrying resistance to blackleg PG4-type isolate through molecular cytogenetic study and application of doubled haploid (DH) technology (keep the original proposal of achieving this through self-pollination, as backup).
- As mentioned above, the second objective of the original project 2012F161R, i.e. 'recurrent backcrossing for introgression of PG4-type resistance from the B-genome of *B. carinata* into the A/C-genome of *B. napus*' is very uncertain due to possible

involvement of more than one gene in the control of this trait and challenge associated with introgression of a gene from the B-genome into the A/C genome. Therefore, for the second objective, recurrent backcrossing was planned, however, with relatively lower emphasis until the B-genome chromosomes carrying resistance is identified.

As reviewed above, the Canadian canola breeders has been utilizing the PG2-type resistance conferred by *Rlm3*/*LmR1* genes (introduced from Australian *B. napus* canola) located on the chromosome A7. To diversify the PG2-type resistance in canola, some of the populations developed in this project were tested for resistance to a PG2-type isolate #3356 for the development of resistant lines carrying genome content introgressed from *B. carinata* (B chromosome). This piece of work was not included in the research proposal; however, included during the course of the research project for greater utilization of the resources developed in this project.

Materials and methods

Plant materials

The plant materials used in this research project comprised 108 advanced backcrossed and self-pollinated families derived from the F_1's of *B. carinata* (acc. #98-14513) × *B. napus* (cv. Westar) and *B. napus* (cv. Polo) × *B. carinata* (acc. #98-14513) interspecific crosses backcrossed to cv. Westar. These families were developed in the research project "#2007F068R", and include 3 BC_1S_1, 5 BC_2, 23 BC_2S_2, 15 BC_3, 44 BC_3S_1 and 18 BC_4 families (Table 1). Seeds of these lines served as starting materials for the development of DH lines for identification of the B genome chromosomes carrying resistance to the *L. maculans* PG4- and PGt-type isolates.

Development of doubled haploid (DH) lines

Microspore culture technique as described by Kebede et al. (2010) was applied on the resistant plants of the above-mentioned families for the development of DH lines carrying B genome chromosome(s).

Self-pollination of the DH-donor plants

Plants that showed resistance to #290CDN (see below for detail) both at cotyledon and adult plant stages and used as donor for production of DH lines were self-pollinated and seeds were harvested. These populations were evaluated for resistance to #290CDN as well as BL05-08RK (PGt-type isolate).

***Leptosphaeria maculans* culture and inoculum preparation**

Two *L. maculans* isolates #290CDN (single spore isolate, PG4 type reaction) and #BL05-08RK (single spore isolate, PGt type reaction) were used for all inoculation experiments. The isolates were cultured on V8 vegetable juice agar plates containing 40mg/L Rose Bengal and 100mg/L streptomycin sulphate (Dhingra & Sinclair, 1985). The culture was maintained under 12-hour photoperiod at 21°C in a NuAire™ incubator (Fernbrrok Lane, MN, USA). After 16 days, each plate was flooded with 5 mL sterile distilled water and the culture surface gently scraped with a sterile metal rod to manually dislodge the pycnidiospores. The pycnidiospore suspensions were then filtered through sterile cheesecloth. The concentration of the spore suspensions were determined using a haemocytometer, and adjusted to 1×10^7 pycnidiospores/mL. The prepared inoculum was used immediately or stored at $+4^oC$ for not more than 24 hours until use.

In addition to the above-mentioned two isolates, a PG2-type isolate #3356 was also used to inoculate some of the populations developed in this research project.

Inoculation tests for blackleg resistance

Screening for reaction to *L. maculans* PG4-type isolate #290CDN was carried out by inoculating 8 to 25 seedlings from each of the advanced backcrossed and self-pollinated lines developed in project "#2007F068R" (Table 1). In addition, *L. maculans* PGt-type isolate #BL05-08RK was used in few of the inoculation experiments to find out if the tested lines also possess resistance against other virulent strains of *L. maculans*.

The DH lines and pedigree families were tested for resistance to #290CDN, #BL05-08RK and #3356. For this, seven-day-old seedlings with fully expanded cotyledons were wounded on each lobe and inoculated with *L. maculans* spore suspension as described by

Sjodin and Glimelius (1989). Disease symptoms were rated 10 to 13 days after inoculation for cotyledon reaction. To screen for blackleg resistance in adult plants, stem-base inoculation of the cotyledon inoculated plants was carried out with 10 µL of 10^7/ml pycnidiospores on plants at the 6-8 leaf stage (Bansal et al. 1994; Sjodin & Glimelius 1989). The plants were then left to grow and evaluated for stem canker at maturity by cutting stems of the double inoculated plants. *Brassica carinata* was used as the resistant control while *B. napus* cultivars Westar and Polo were used as the susceptible controls. The severity of the disease symptoms at the cotyledon stage was scored based on the 0 - 9 scale devised by Delwiche (1980), while the severity of the symptoms at the adult stage was determined based on the 0 - 5 scale proposed by Western Canada Canola/Rapeseed Recommending Committee (WCC/RRC). Based on mean scores for cotyledon infection, the plants were grouped as resistant (score ≤ 1) intermediate (score > 1 to ≤ 4), and susceptible (score > 4); while based on mean scores for internal necrosis at adult stage, the plants were grouped as resistant (score ≤ 1), intermediate (score >1 to ≤ 2), and susceptible (score > 2).

Inoculations experiments were repeated with some of the DH lines found to be resistant to the PG4- and PGt-type isolates at either the cotyledon and/or adult plant stages in the initial screening.

DNA isolation and SSR marker analysis for identification of B genome chromosomes

Young leaves were collected from each plant, freeze dried and stored at -80 ^{o}C until used. DNA isolation was carried out using the GENELUTE Plant Genomic DNA Kit (Sigma-Aldrich, St. Louis, CA, USA) according to the manufacturer's instructions. The quality of the DNA was evaluated by spectrometry using the 260/280 nm absorbance ratio method, and the DNA concentration estimated at 260 nm with an ND-1000 Nanodrop spectrophotometer (Nanodrop Technologies, Inc., Wilmington, DE, USA).

Molecular cytogenetic analysis was done by the use of simple sequence repeat (SSR) markers that we identified in project "#2007F068R" to be co-segregating with the B genome chromosomes. To confirm the presence of B genome chromosomes other than those known to be present in the donor parents, two SSR markers from each of the B1 to B8 were tested on the DH lines. Two randomly selected samples (plants) of each DH line were genotyped to study the association of B genome chromosomes with resistance.

Using the genomic DNA from the advanced backcrossed and self-pollinated families and the DH lines as template, PCR was carried out in a total volume of 13 µL containing 1x Taq buffer, 2.0 mM $MgCl_2$, 200 µM dNTPs, 0.4 µM forward primer modified at the 5'-end with an M13 tail, 0.4 µM reverse primer, 0.2 µM fluorescently labeled M13 primer, and 1.25U Taq polymerase (Promega Corp., Madison, WI, USA). The reactions were carried out in an MJ Research PTC-200 DNA Engine Thermal Cycler (Bio-Rad laboratories, Hercules, CA, USA); amplifications consisted of an initial denaturation step of 5 min at 94 °C, 35 cycles of 1 min at 94 °C, 1 min at optimum annealing temperature of each primer, and 1.5 min at 72 °C, followed by a final extension of 30 min at 72 °C.

The amplified products from four reactions, each labeled with one of the four fluorescent dyes FAM, VIC, NED, and PET, were pooled and analyzed by capillary electrophoresis on an ABI PRISM 3730xl DNA analyzer (Life Technologies, USA) or through electrophoresis on 12% polyacrylamide gels when the alleles at an SSR locus differed by more than 25 bp. The amplified fragments were visualized after staining with SYBR® Safe DNA Gel Stain (Life Technologies, USA). To confirm the reproducibility of molecular data, we repeated PCR and genotyping in about 10% of the samples.

Recurrent backcrossing for introgression of resistance into *B. napus*

As mentioned in the revised objectives, due to challenge of introgression of resistance and possible involvement of multiple genes controlling resistance to PG4-type isolate, relatively lower emphasis was given on recurrent backcrossing, and gave major emphasis on identification of self-pollinated plants carrying resistance to #290CDN/#BL05-08RK.

Results

Evaluation of the advanced backcrossed and self-pollinated generation plants for resistance to PG4- and PGt-type isolates #290CDN and #BL05-08RK

A total of 820 and 237 different backcross generation plants from 108 families were screened for resistance to *L. maculans* PG4-type isolate #290CDN and PGt-type isolate #BL05-08RK,

respectively. The number of plants from each generation screened and their resistances to these two isolates are shown in Table 1.

In the case of the plants screened with #290CDN, 28 of the 820 plants were found to be resistant, 32 intermediate and 760 susceptible at the cotyledon stage. The resistant parent *B. carinata* always had disease score ≤1. Thus, only 3.4 % of the seedlings were found to carry cotyledon resistance despite selection for resistance being carried out in each generation while developing these families. In the case of the plants screened with *L. maculans* #BL05-08RK, 11 (4.6 %) of the 237 plants were resistant, 9 intermediate and 217 susceptible. This indicates that cotyledon resistance to the PG4 and PGt-type *L .maculans* isolates in *B. carinata* is under similar genetic control. This is also evident from the study conducted with the DH population challenged with these two isolates (see below under sub head 'Relationship between resistance to #290CDN and #BL05-08RK at cotyledon and adult plant stages').

Quantitative assessment of adult plant resistance to *L. maculans* #290CDN could not be carried out in 27 of the 820 plants because these plants died before maturity. As a result, a total of 793 plants were examined at the adult plant stage, where 187 (23.6%) were resistant, 124 intermediate and 482 susceptible. In the case of adult plant resistance to *L. maculans* #BL05-08, 235 plants of the 237 could be scored. Forty nine (20.9%) of the 235 plants were resistant, 13 intermediate and 173 susceptible. About 4% of the susceptible control *B. napus* cvs. Westar and Polo also showed resistance at adult plant stage - might be due to disease escape. However, adult plant resistance was found in a far greater number of plants (23.6 % for *L. maculans* #290CDN and 20.9% for #BL05-08) as compared to cotyledon resistant plants in these advanced backcross and self-pollinated families. This suggests that cotyledon and adult plant resistances to the isolate are under different genetic controls. Cotyledon resistance to isolate #290CDN was observed in two BC_2S_2, two BC_3, four BC_3S_1, and one BC_4 families while adult stage resistance was found in plants from all 108 families screened, but predominantly in the BC_3 and BC_4 families (Table 1).

A total of 23 plants from two BC_2S_2, one BC_4 and 11 BC_3 generations were crossed to the susceptible cultivar Westar. At the adult stage 10 plants were resistant, six intermediate and seven susceptible. The F_1 seeds harvested from the 10 plants showing resistance to #BL05-08RK or #290CDN at adult stage were retained for further study.

Table 1. Cotyledon and adult plant resistance responses to Leptosphaeria maculans PG4-type isolate #290CDN and PGt-type isolate #BL05-08RK in 71 advanced backcrossed and self-pollinated families derived from *Brassica napus* × *B. carinata* interspecific cross.

Generation	No. fam	Phenotypic reaction	Seeding 1: Inoculated with #290CDN			Seeding 2: Inoculated with #290CDN			Seeding 3: Inoculated with #290CDN			Seeding 3: Inoculated with #BL05-08RK			Seeding 4: Inoculated with #290CDN			Seeding 5: Inoculated with #290CDN			Seeding 5: Inoculated with BL05-08			Total Seeding 1-5			**Total**
			R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	
BC_1S_1	3	Cotyledon	-	-	-	-	-	-	-	-	-	-	-	-	0	0	9	-	-	-	-	-	-	0	0	9	**9**
		Adult	-	-	-	-	-	-	-	-	-	-	-	-	0	0	9	-	-	-	-	-	-	0	0	9	**9**
BC_2	5	Cotyledon	-	-	-	-	-	-	-	-	-	-	-	-	0	0	19	-	-	-	-	-	-	0	0	19	**19**
		Adult	-	-	-	-	-	-	-	-	-	-	-	-	3	5	10	-	-	-	-	-	-	3	5	10	**18**
BC_2S_2	23	Cotyledon	0	0	81	-	-	-	-	-	-	-	-	-	12	16	24	0	0	16	0	0	16	12	16	137	**165**
		Adult	13	10	46	-	-	-	-	-	-	-	-	-	16	7	29	2	2	12	4	1	11	35	20	98	**153**
BC_3	15	Cotyledon	-	-	-	1	0	105	8	6	46	11	9	41	1	3	59	-	-	-	-	-	-	21	18	251	**290**
		Adult	-	-	-	57	20	29	17	11	32	22	6	33	13	15	35	-	-	-	-	-	-	109	52	129	**290**
BC_3S_1	44	Cotyledon	0	0	85	-	-	-	-	-	-	-	-	-	4	6	25	0	0	152	0	0	152	4	6	414	**424**
		Adult	5	9	69	-	-	-	-	-	-	-	-	-	4	10	13	14	9	127	19	6	125	42	34	334	**410**
BC_4	18	Cotyledon	0	0	104	-	-	-	-	-	-	-	-	-	2	1	27	0	0	8	0	0	8	2	1	147	**150**
		Adult	30	18	55	-	-	-	-	-	-	-	-	-	11	6	12	2	2	4	4	0	4	47	26	75	**148**
Total	**108**	**Cotyledon**	**0**	**0**	**270**	**1**	**0**	**105**	**8**	**6**	**46**	**11**	**9**	**41**	**19**	**26**	**163**	**0**	**0**	**176**	**0**	**0**	**176**	**39**	**41**	**977**	**1057**
		Adult	**48**	**37**	**170**	**57**	**20**	**29**	**17**	**11**	**32**	**22**	**6**	**33**	**47**	**43**	**108**	**18**	**13**	**143**	**27**	**7**	**140**	**236**	**137**	**655**	**1028**

NB: Adult stage resistance could not be determined for all plants as some died before scoring.
R = resistant, I = intermediate, S = susceptible

Identification of donor plants, based on resistance to PG4- and PGt-type isolates #290CDN and #BL05-08RK, for DH production

Thirteen plants from seven families derived from (*B. carinata* × Westar) × Wester, resistant to *L. maculans* PG4-type isolate #290CDN at both cotyledon and adult plant stages during "seeding 4", were used for DH production. These comprised eight plants from three BC_2S_2 families (5CA1250.084p1, 5CA1250.084p2, 5CA1250.084p4; 5CA1250.085p2; 5CA1250.088p1, 5CA1250.088p2, 5CA1250.088p3 and 5CA1250.088p4), three plants from three BC_3S_1 families (5CA1250.078p2, 5CA1250.079p1 and 5CA1250.082p3), and two plants from one BC_4 family (5CA1250.093p1 and 5CA1250.093p4).

In the case of the families derived from (Polo × *B. carinata*) × Wester, eight plants from two BC_3 families were found to be resistant to *L. maculans* PG4-type isolate #290CDN at both cotyledon and adult plant stages during "seeding 3". Four plants from each of the two families (5CA1341.072p33, 5CA1341.072p34, 5CA1341.072p35, 5CA1341.072p41; 5CA1341.073p25, 5CA1341.073p26, 5CA1341.073p27 and 5CA1341.073p28) were used for DH production.

Thus, a total of eight plants from "seeding 3" and 13 plants from "seeding 4" from BC_2S_2, BC_3, BC_3S_1 and BC_4 that showed resistance at both cotyledon and adult stages to #290CDN were used for DH production (Table 2). In spite of numerous attempts, DH production could not be carried out on seven of the 21 resistant plants. It was observed that DH production was more difficult from the plants belonging to the BC_3 family 5CA1341.073 than from 5CA1341.072.

In the case of the BC_3 plants screened with *L. maculans* PGt-type isolate #BL05-08RK during "seeding 3", nine of the 11 plants found to be resistant at both the cotyledon and adult stages were used for DH production (Table 3). The remaining two plants became susceptible at the adult stage and died before maturity. Eight of the nine plants in which DH production was successful were of the genotype 5CA1341.072 while DH production was successful in only one plant of the genotype 5CA1341.073. Thus, the efficiency of the DH production depended largely on the genotype. The number DH lines produced from different BC_2S_2, BC_3, BC_3S_1 and BC_4 plants are presented in Table 2 and 3.

Identification of B genome chromosomes in the donor plants used for DH production by use of SSR markers

To identify B genome chromosomes from *B. carinata* present in the cotyledon and adult plant resistant lines, molecular marker analysis was carried out with 26 B genome specific SSR markers on the 27 (Table 2 and 3) of the 39 (Table 1) resistant BC_2S_2, BC_3, BC_3S_1 and BC_4 donor (DH) plants. Data showed that 10 plants carried only the B3 (J18) and one carried only the B6 (J14) chromosome; while 9 plants carried the B6 (J14) and B3 (J18) chromosomes from *B. carinata*. One resistant plant was found to carry only the B7 (J13) chromosome while three resistant plants were found to carry B7 (J13) and B1 (J17) chromosomes. One resistant plant was found to carry three B genome chromosomes, B7 (J13), B2 (J15) and B1 (J17). SSR marker analysis also revealed that the BC_3 plants belonging to the family 5CA1341.072 contained either B3 (J18) or B6 (J14) and B3 (J18) chromosomes, while plants belonging to the family 5CA1341.073 contained only B7 (J13) or B7 (J13) and B1 (J17) or B7 (J13), B2 (J15) and B1 (J17) chromosomes.

Table 2. Production of DH lines from advanced backcross and self-pollinated generation plants of (*B. carinata* accession #98-14513 × *Brassica napus* cv. Westar) × *B. napus* cv. Westar (CWW) and (*Brassica napus* cv. Polo × *B. carinata* accession #98-14513) × *B. napus* cv. Westar (PCW) showing resistance to *Leptosphaeria maculans* PG4-type isolate #290CDN at cotyledon and adult plant stages.

Pedigree	Mother plant gen.	Mother plant	Gene-ration	Family name & plant no.	B genome chromosome in DH donor plant	DH population	No. embryos to solid media	No. seedlings to soil	No. DH lines produced
PCW	BC_2S_1	5CA1341.052p5	BC_2S_2	5CA1341.084p1	B3, B6	Popl#598	160	28	2
PCW	BC_2S_1	5CA1341.052p5	BC_2S_2	5CA1341.084p2	B3	Popl#599	504	181	36
PCW	BC_2S_1	5CA1341.052p5	BC_2S_2	5CA1341.084p4	B3, B6	Popl#601	240	82	14
PCW	BC_2S_1	5CA1341.052p13	BC_2S_2	5CA1341.085p2	-	Popl#603	-	-	-
CWW	BC_2S_1	5CA1250.059p14	BC_2S_2	5CA1250.088p1	B6, B6	Popl#361	620	281	95
CWW	BC_2S_1	5CA1250.059p14	BC_2S_2	5CA1250.088p2	-	Popl#362	-	-	-
CWW	BC_2S_1	5CA1250.059p14	BC_2S_2	5CA1250.088p3	B6	Popl#363	395	124	27
CWW	BC_2S_1	5CA1250.059p14	BC_2S_2	5CA1250.088p4	B6	Popl#364	112	31	0
PCW	BC_2	5CA1341.018-p2	BC_3	5CA1341.072p33	B3	Popl#566	67	19	14
PCW	BC_2	5CA1341.018-p2	BC_3	5CA1341.072p34	B3, B7	Popl#567	346	49	2
PCW	BC_2	5CA1341.018-p2	BC_3	5CA1341.072p35	B3, B7	Popl#568	703	268	79
PCW	BC_2	5CA1341.018-p2	BC_3	5CA1341.072p41	B3	Popl#569	830	55	11
PCW	BC_2	5CA1341.018p12	BC_3	5CA1341.073p25	B1, B7	Popl#562	-	-	-
PCW	BC_2	5CA1341.018p12	BC_3	5CA1341.073p26	B1, B2, B7	Popl#563	316	102	5
PCW	BC_2	5CA1341.018p12	BC_3	5CA1341.073p27	B1, B7	Popl#564	4	-	-
PCW	BC_2	5CA1341.018p12	BC_3	5CA1341.073p28	B7	Popl#565	-	-	-
CWW	BC_3	5CA1250.039p7	BC_3S_1	5CA1250.078p2	B3	Popl#322	308	107	27
CWW	BC_3	5CA1250.039-p9	BC_3S_1	5CA1250.079p1	-	Popl#325	-	-	-
CWW	BC_3	5CA1250.051-p8	BC_3S_1	5CA1250.082p3	B3, B6	Popl#339	1	-	0
CWW	BC_3	5CA1250.039p14	BC_4	5CA1250.093p1	B3	Popl#381	486	194	76
CWW	BC_3	5CA1250.039p14	BC_4	5CA1250.093-p4	B3	Popl#384	172	80	29
			Total				**5264**	**1601**	**417**

Table 3. Production of DH lines from advanced backcross generation plants of (*Brassica napus* cv. Polo × *B. carinata* accession #98-14513) × *B. napus* cv. Westar (PCW) showing resistance to *Leptosphaeria maculans* PGt-type isolate #BL05-08RK at cotyledon and adult plant stages.

Pedigree	Mother plant gen.	Mother plant	Gene-ration	Family name & plant no.	B genome chromosome in DH donor plant	DH population	No. embryos to solid media	No. seedlings to soil	No. DH lines produced
PCW	BC_2	5CA1341.018p2	BC_3	5CA1341.072p9	B3, B6	Popl#553	91	2	1
PCW	BC_2	5CA1341.018p2	BC_3	5CA1341.072p10	B3	Popl#554	464	243	45
PCW	BC_2	5CA1341.018p2	BC_3	5CA1341.072p11	B3	Popl#555	698	142	13
PCW	BC_2	5CA1341.018p2	BC_3	5CA1341.072p12	B3, B6	Popl#556	12	1	1
PCW	BC_2	5CA1341.018p2	BC_3	5CA1341.072p13	B3, B6	Popl#557	66	11	0
PCW	BC_2	5CA1341.018p2	BC_3	5CA1341.072p14	B3	Popl#558	340	156	21
PCW	BC_2	5CA1341.018p2	BC_3	5CA1341.072p17	B3, B6	Popl#559	20	8	0
PCW	BC_2	5CA1341.018p2	BC_3	5CA1341.072p18	B3, B6	Popl#560	826	271	163
PCW	BC_2	5CA1341.018p12	BC_3	5CA1341.073p9	B1, B3	Popl#552	109	22	0
		Total					**2626**	**855**	**244**

Evaluation of the 355 DH lines for resistance to PG4- and PGt-type isolates #290CDN and #BL05-08RK

A total of 661 (417 + 244) DH lines were produced from the donor plants derived from (*B. carinata* × Westar) × Wester and (Polo × *B. carinata*) × Westar crosses (Table 2 and 3).

In the first phase of this project, 355 of the 661 DH lines were evaluated for resistance to *L. maculans* PG4-type isolate #290CDN and PGt-type isolate #BL05-08RK. For this, six seedlings of each line were challenged with each of these two isolates for cotyledon resistance. Thus, a total of 4,081 seedlings were inoculated with the *L. maculans* isolates at the seedling stage for cotyledon resistance. The number of plants from each of the DH lines screened and their resistances to the isolates is presented in Table 4. Out of 2,039 plants screened for resistance to #290CDN at cotyledon stage, 0.74 % (15/2,039) were scored as resistant (R), 0.49 % (10/2,039) intermediate resistant (I) and 98.8 % (2,014/2,039) susceptible (S). One hundred and eighty four plants died before maturity and hence could not be scored for adult resistance. At the adult stage, 20.9 % (387/1,855) and 8.7 % (162/1,855)

of the plants, respectively, were resistant and intermediate-resistant, while 70.4 % (1,306/1,855) were susceptible to this isolate. In the case of the DH lines screened for resistance to PGt isolate #BL05-08RK, a total of 2,042 plants were screened for cotyledon resistance, of which 0.59 % (12/2,042) were resistant, 0.93 % (19/2,042) intermediate and 98.5 % (2,011/2,042) susceptible (S). At the adult stage, 22.7 % (408/1,795) and 9.2 % (166/1,795) of the plants, respectively, were resistant and intermediate resistant, while 68.0 % (1,221/1,795) were susceptible.

Thus, at the cotyledon stage about 1.2 - 1.5 % of the plants screened were resistant or intermediate while at the adult stage approximately 30 % of the plants screened were resistant or intermediate (Figure 1; Table 4). Cotyledon resistance was confirmed in two DH lines of Popl#554 and Popl#558. The number of DH lines carrying resistance to the two isolates is presented in Figure 2.

Evaluation of the 40 pedigree families for resistance to PG4- and PGt-type isolates #290CDN and #BL05-08RK

In the first phase of testing, 40 BC_4S_1 generation pedigree families comprising six and 34 families derived, respectively, from the (*B. carinata* × Westar) × Wester and (Polo × *B. carinata*) × Wester were screened for reaction to both *L. maculans* isolates #BL05-08RK and #290CDN (Table 5). A total of 480 seedlings comprising 12 seedlings from each of the families were screened against the isolates at the cotyledon stage; where 12.5% (60/480) of the seedlings were resistant, 2.7 % (13/480) intermediate resistant and the remaining 84.8 % (407/480) were susceptible. All plants from two families (5CA1341.556-A1263, 5CA1341.937-A1263) were completely resistant, plants from 11 families (5CA1341.554-A1253, 5CA1341.555-A1253, 5CA1341.559-A1253, 5CA1341.560- A1253, 5CA1341.561-A1253, 5CA1341.562-A1253, 5CA1341.566-A1253, 5CA1341.567-A1253, 5CA1341.568-A1253, 5CA1341.941-1263, and 5CA1250393-A1263) were segregating for resistance, one family (5CA1341.563-A1253) was intermediate-resistant and all plants from the remaining 26 families were susceptible to both isolates at the cotyledon stage.

Quantitative assessment of adult stage resistance was carried out on 324 plants from these 40 families. This is because 156 (32.5%) plants died before maturity. Of the surviving

plants, 16.4 % (53/324) of the plants were resistant at the adult stage to either of these two isolates, 12.0 % (39/324) intermediate-resistant and 71.6 % (232/324) susceptible. Ten plants from five families were resistant at both the cotyledon and adult stages; however, four plants failed to produce seed. The remaining six plants comprised two from the family 5CA1341.937-A1263 (p1 and p5) showing resistance to #CDN290, and one plant from 5CA1341.560-A1253 (p7), one from 5CA1341.562-A1253 (p8) and two from 5CA1341.568-A1253 (p9, p10) showing resistance to #BL05-08RK. In addition, about 8.9% of the cotyledon susceptible plants became resistant at the adult stage while 10.5% of the cotyledon resistant plants became susceptible at the adult stage. Analysis of the phenotypic data of the pedigree populations also suggest that resistance to *L. maculans* isolates #BL05-08RK and #290CDN is under complex genetic control.

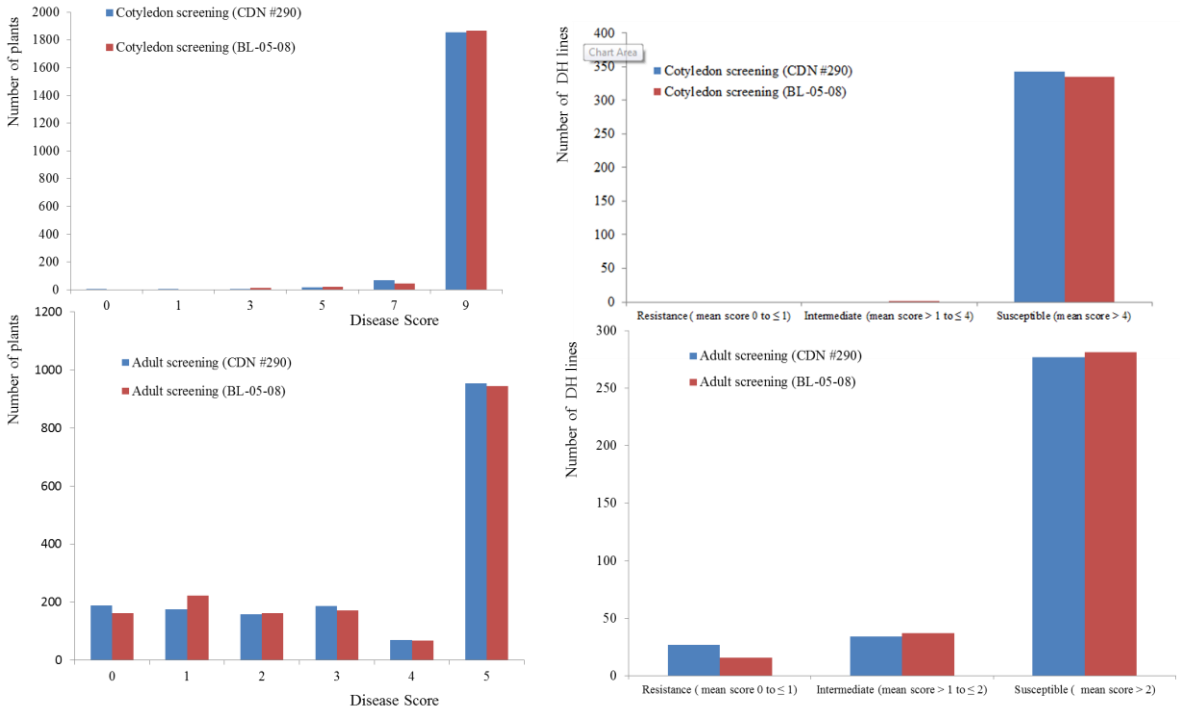

Fig 1. (A) Number of individual plants of 355 DH lines showing cotyledon (*upper*) and adult-plant (*lower*) resistance to *L. maculans* PG4-type isolate #290CDN and PGt-type isolate #BL05-08. (B) Number of DH lines carrying cotyledon (*upper*) and adult-plant (*lower*) resistance to *L. maculans* PG4-type isolate #290CDN and PGt-type isolate #BL05-08

Table 4. Cotyledon and adult plant responses to *L. maculans* PG4-type isolate #290CDN and PGt-type isolate #BL05-08RK in 355 doubled haploid (DH) lines derived from (*B. carinata* accession #98-14513 × *Brassica napus* cv. Westar) × *B. napus* cv. Westar (CWW) and (*Brassica napus* cv. Polo × *B. carinata* accession #98-14513) × *B. napus* cv. Westar (PCW) interspecific crosses. DH donor plants and their B genome chromosome contents also presented. Data for cotyledon and adult resistance indicate number of seedlings/plants.

DH donor plants			DH population	# DH lines prod-uced	No.DH lines screened	Total no.of plants screened	*L. maculans* PG4 isolate #290CDN							*L. maculans* PGt isolate #BL05-08RK						
							Cotyledon resistance			Adult resistance				Cotyledon resis.			Adult resistance			
Ped.	Gen.	Chrom.					R	I	S	R	I	S	D	R	I	S	R	I	S	D
PCW	BC_3	B3, B6	Popl#553	1	1	12	0	0	6	0	0	4	2	0	0	6	0	0	5	1
PCW	BC_3	B3	Popl#554	45	21	252	0	0	123	33	5	50	35	0	1	128	27	5	58	39
PCW	BC_3	B3	Popl#555	13	7	84	0	6	36	0	4	10	28	0	6	36	0	0	15	27
PCW	BC_3	B3	Popl#556	1	1	12	0	0	6	1	1	4	0	0	0	6	2	1	3	0
PCW	BC_3	B3	Popl#558	21	12	142	1	1	69	13	6	27	25	0	1	70	17	5	26	23
PCW	BC_3	B3, B6	Popl#560	163	129	1482	1	0	744	187	86	470	2	0	0	737	182	88	413	54
PCW	BC_3	B3, B7	Popl#567	2	1	12	0	0	6	0	0	2	4	0	0	6	0	0	6	0
PCW	BC_3	B3, B7	Popl#568	79	64	721	1	3	356	75	24	207	54	0	11	350	87	32	192	50
PCW	BC_3	B3	Popl#569	11	5	60	12	0	18	2	0	20	8	12	0	18	3	3	17	7
PCW	BC_2S_2	B3	Popl#599	36	4	44	0	0	21	5	2	10	4	0	0	23	8	1	10	4
PCW	BC_2S_2	B3, B6	Popl#601	14	2	24	0	0	12	0	4	8	0	0	0	12	3	2	7	0
CWW	BC_3S_1	B3	Popl#322	27	10	115	0	0	58	14	6	35	3	0	0	57	17	4	32	4
CWW	BC_2S_2	B3, B6	Popl#361	95	43	495	0	0	246	24	15	192	15	0	0	249	29	12	186	22
CWW	BC_2S_2	B3	Popl#363	27	12	137	0	0	67	15	6	45	1	0	0	70	11	4	53	2
CWW	BC_4	B3	Popl#381	76	32	367	0	0	185	17	3	165	0	0	0	182	21	6	143	12
CWW	BC_4	B3	Popl#384	29	11	122	0	0	61	1	0	57	3	0	0	61	1	3	55	2
Total					**355**	**4081**	**15**	**10**	**2014**	**387**	**162**	**1306**	**184**	**12**	**19**	**2011**	**408**	**166**	**1221**	**247**

Cotyledon reaction: R = resistant (disease score 0 to ≤ 1), I = intermediate (score > 1 to ≤ 4), and S = susceptible (score > 4).
Adult reaction: R = resistant (disease score ≤ 1), I = intermediate (score > 1 to ≤ 2), S = susceptible (score > 2), and D = dead (plants died before maturity

Table 5. Cotyledon and adult plant responses to *L. maculans* PG4-type isolate #290CDN and PGt-type isolate #BL05-08RK in 40 pedigree lines derived from (*B. carinata* accession #98-14513 × *Brassica napus* cv. Westar) × *B. napus* cv. Westar (CWW) and (*Brassica napus* cv. Polo × *B. carinata* accession #98-14513) × *B. napus* cv. Westar (PCW) interspecific crosses.

Pedigree	Gen	Pedigree family	*L. maculans* PG4 isolate #290CDN							*L. maculans* PGt isolate #BL05-08RK						
			Cotyledon resistance			Adult resistance*				Cotyledon resistance			Adult resistance*			
			R	I	S	R	I	S	D	R	I	S	R	I	S	D
CWW	BC_4S_1	5CA1250.393-A1263	0	0	6	0	5	1	0	1	0	5	1	0	5	0
CWW	BC_4S_1	5CA1250.394-A1263	0	0	6	0	0	1	5	0	0	6	0	0	6	0
CWW	BC_4S_1	5CA1250.395-A1263	0	0	6	0	0	6	0	0	0	6	0	0	3	3
CWW	BC_4S_1	5CA1250.396-A1263	0	0	6	0	0	3	3	0	0	6	0	0	2	4
CWW	BC_4S_1	5CA1250.397-A1263	0	0	6	0	0	3	3	0	0	6	1	0	5	0
CWW	BC_4S_1	5CA1250.398-A1263	0	0	6	0	0	4	2	0	0	6	0	1	3	2
PCW	BC_4S_1	5CA1341.940-A1263	0	0	6	2	0	2	2	0	0	6	0	0	5	1
PCW	BC_4S_1	5CA1341.941-A1263	0	0	6	0	0	2	4	1	0	5	0	0	2	4
PCW	BC_4S_1	5CA1341.942-A1263	0	0	6	1	1	3	1	0	0	6	1	0	2	3
PCW	BC_4S_1	5CA1341.923-A1263	0	0	6	0	0	1	5	0	0	6	0	0	1	5
PCW	BC_4S_1	5CA1341.924-A1263	0	0	6	3	1	0	2	0	0	6	2	0	4	0
PCW	BC_4S_1	5CA1341.925-A1263	0	0	6	2	0	2	2	0	0	6	1	0	4	1
PCW	BC_4S_1	5CA1341.926-A1263	0	0	6	2	1	2	1	0	0	6	1	2	2	1
PCW	BC_4S_1	5CA1341.927-A1263	0	0	6	1	2	1	2	0	0	6	0	0	2	4
PCW	BC_4S_1	5CA1341.928-A1263	0	0	6	3	1	1	1	0	0	6	0	3	3	0
PCW	BC_4S_1	5CA1341.929-A1263	0	0	6	0	2	1	3	0	0	6	0	2	3	1
PCW	BC_4S_1	5CA1341.930-A1263	0	0	6	2	0	4	0	0	0	6	0	0	3	3
PCW	BC_4S_1	5CA1341.931-A1263	0	0	6	0	0	3	3	0	0	6	0	0	4	2
PCW	BC_4S_1	5CA1341.932-A1263	0	0	6	1	0	2	3	0	0	6	0	0	4	2
PCW	BC_4S_1	5CA1341.933-A1263	0	0	6	0	0	1	5	0	0	6	2	1	2	1
PCW	BC_4S_1	5CA1341.934-A1263	0	0	6	0	1	3	2	0	0	6	0	1	4	1
PCW	BC_4S_1	5CA1341.935-A1263	0	0	6	1	0	2	3	0	0	6	0	0	3	3
PCW	BC_4S_1	5CA1341.936-A1263	0	0	6	1	1	3	1	0	0	6	0	1	4	1
PCW	BC_4S_1	5CA1341.937-A1263	6	0	0	2	0	1	3	6	0	0	0	0	5	1
PCW	BC_4S_1	5CA1341.938-A1263	0	0	6	0	0	4	2	0	0	6	0	2	3	1
PCW	BC_4S_1	5CA1341.939-A1263	0	0	6	2	0	4	0	0	0	6	0	0	4	2
PCW	BC_4S_1	5CA1341.553-A1263	0	0	6	0	0	3	3	0	0	6	0	0	3	3
PCW	BC_4S_1	5CA1341.554-A1263	2	0	4	0	0	4	2	2	0	4	0	0	3	3
PCW	BC_4S_1	5CA1341.555-A1263	3	0	3	1	1	2	2	2	0	4	0	0	6	0
PCW	BC_4S_1	5CA1341.556-A1263	6	0	0	0	0	3	3	6	0	0	0	0	3	3
PCW	BC_4S_1	5CA1341.559-A1263	2	0	4	0	0	3	3	3	0	3	0	1	4	1
PCW	BC_4S_1	5CA1341.560-A1263	0	0	6	2	0	4	0	3	0	3	2	2	0	2
PCW	BC_4S_1	5CA1341.561-A1263	2	0	4	1	0	4	1	1	1	4	3	0	2	1
PCW	BC_4S_1	5CA1341.562-A1263	2	3	1	1	1	4	0	3	0	3	2	0	2	2
PCW	BC_4S_1	5CA1341.563-A1263	0	5	1	0	0	5	1	0	4	2	0	0	2	4

Pedigree	Gen	Pedigree family	*L. maculans* PG4 isolate #290CDN							*L. maculans* PGt isolate #BL05-08RK						
			Cotyledon resistance			Adult resistance*				Cotyledon resistance			Adult resistance*			
			R	I	S	R	I	S	D	R	I	S	R	I	S	D
PCW	BC_4S_1	5CA1341.566-A1263	0	0	6	1	0	2	3	3	0	3	0	0	4	2
PCW	BC_4S_1	5CA1341.567-A1263	2	0	4	0	0	3	3	0	0	6	0	1	2	3
PCW	BC_4S_1	5CA1341.568-A1263	3	0	3	1	1	3	1	1	0	5	2	0	3	1
PCW	BC_4S_1	5CA1341.569-A1263	0	0	6	4	0	1	1	0	0	6	0	3	3	0
Total			**28**	**8**	**204**	**35**	**19**	**103**	**83**	**32**	**5**	**203**	**18**	**20**	**129**	**73**

Cotyledon reaction: R = resistant (disease score 0 to ≤ 1), I = intermediate (score > 1 to ≤ 4), and S = susceptible (score > 4).
Adult reaction: R = resistant (disease score ≤ 1), I = intermediate (score > 1 to ≤ 2), S = susceptible (score > 2), and D = dead (plants died before maturity.

Identification of B genome chromosomes in the DH lines by use of SSR markers

A total of 113 plants (mainly resistant) belonging to 51 DH lines were tested with 16 SSR markers − two from each of the eight B genome chromosomes (B1 to B8 or J11 to J18). Agarose gel electrophoresis of PCR products of these plants is presented in Supplementary Figures 1 to 20 (Appendix 1), and the genotypic analysis is summarized in Table 6. Sixteen plants contained the B3 (J18) chromosome (Figures S4, S19 and S20) while only one plant contained the B1 (J17) chromosome (Figures S4, S17 and S18). In the case of the B7 (J13) chromosome SSR markers, traces of the PCR products of the same size as the expected product of *B. carinata* were found in 53 of the 113 DH plants (Figure S2 and S9), and traces of one SSR marker of the B8 (J12) chromosome (Figures S1 and S7) of the expected product together with several non-specific products were found in six of the 113 DH plants. Of the 113 plants, 83 showed resistance to 290CDN/BL05-08 at adult plant stage (score 0-1) where 42% (35/83) found to carry B7 chromosome. The occurrence of B7 chromosome in such high proportion of the resistant plants indicates that this chromosome might carry gene which is involved in the control of resistance to these isolates. Thirty-one of these DH lines were tested again for confirmation of this resistance (Table 7). Forty-three DH plants lacking B genome chromosomes were also found to be resistant at adult stage (Table 6).

Table 6. Identification of B genome content in individual plants of DH lines derived from advanced backcross generation plants of (*Brassica napus* cv. Polo × *B. carinata* accession #98-14513) × *B. napus* cv. Westar (PCW) and their association with resistance to *L. maculans* PG4-type isolate #290CDN and PGt-type isolate #BL05-08RK

Gel #	DH lines/plants	Adult	B5(J11)		B8 (J12)		B7 (J13)		B6 (J14)		B2 (J15)		B4 (J16)		B1 (J17)		B3 (J18)	
		Score	#503	#507	#508	#511	#515	#519	#520	#524	#528	#531	#533	#535	#541	#543	#544	#547
SP1	*B. napus* cv. Polo	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
RP	*B. carinata*	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SP2	*B. napus* cv. Westar	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	5CA1341.851-p1	1	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
2	5CA1341.859-p7	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	5CA1341.896-p7	0	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
4	5CA1341.899-p7	0	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
5	5CA1341.900-p1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	5CA1341.903-p1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	5CA1341.903-p7	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	5CA1341.574-p1	0	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
9	5CA1341.577-p1	1	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
10	5CA1341.592-p1	0	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
11	5CA1341.596-p1	0	-	-	+	+	-	+	+	+	+	+	-	-	+	+	-	-
12	5CA1341.612-p7	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	5CA1341.621-p7	1	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
14	5CA1341.622-p7	1	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
15	5CA1341.627-p7	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	5CA1341.632-p7	0	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
17	5CA1341.676-p7	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	5CA1341.642-p7	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	5CA1341.702-p7	1	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
20	5CA1341.681-p1	1	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
21	5CA1341.728-p1	1	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
22	5CA1341.745-p6	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
23	5CA1341.754-P1	0	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
24	5CA1341.754-P2	0	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
25	5CA1341.756-p3	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Gel#	DH lines/plants	Adult score	B5 (J11)		B8 (J12)		B7 (J13)		B6 (J14)		B2 (J15)		B4 (J16)		B1 (J17)		B3 (J18)	
			#503	#507	#508	#511	#515	#519	#520	#524	#528	#531	#533	#535	#541	#543	#544	#547
26	5CA1341.756-p5	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	5CA1341.756-p6	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	5CA1341.759-p3	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	5CA1341.759-p4	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
31	5CA1341.759-p5	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	5CA1341.759-p6	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	5CA1341.771-p1	3	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
34	5CA1341.771-p2	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
35	5CA1341.771-p3	0	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
36	5CA1341.771-p4	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
37	5CA1341.771-p6	5	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
38	5CA1341.787-p8	3	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+
39	5CA1341.808-p5	5	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+
40	5CA1341.817-p8	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	5CA1341.817-p9	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	5CA1341.837-p9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	5CA1341.837-p10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	5CA1341.839-p3	0	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
45	5CA1341.839-p4	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	5CA1341.839-p6	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	5CA1341.839-p8	0	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
48	5CA1341.839-p11	0	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
49	5CA1341.839-p12	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
50	5CA1341.843-p3	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
51	5CA1341.843-p4	0	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
52	5CA1341.845-p11	5	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-
53	5CA1341.857-p4	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
54	5CA1341.857-p11	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
55	5CA1341.857-p12	2	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-

Gel#	DH lines/plants	Adult score	B5 (J11)		B8 (J12)		B7 (J13)		B6 (J14)		B2 (J15)		B4 (J16)		B1 (J17)		B3 (J18)	
			#503	#507	#508	#511	#515	#519	#520	#524	#528	#531	#533	#535	#541	#543	#544	#547
57	5CA1341.862-p7	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
58	5CA1341.862-p8	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
59	5CA1341.862-p9	0	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
60	5CA1341.862-p10	0	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
61	5CA1341.863-p2	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
62	5CA1341.863-p4	1	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
63	5CA1341.863-p11	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
64	5CA1341.863-p12	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
65	5CA1341.865-p9	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
66	5CA1341.867-p8	0	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
67	5CA1341.867-p3	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
68	5CA1341.868-p4	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
69	5CA1341.868-p5	5	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
70	5CA1341.868-p6	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
71	5CA1341.868-p7	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
72	5CA1341.868-p8	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
73	5CA1341.868-p9	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
74	5CA1341.868-p11	0	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
75	5CA1341.868-p12	0	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
76	5CA1341.869-p1	2	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
77	5CA1341.869-p2	0	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
78	5CA1341.869-p3	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
79	5CA1341.869-p4	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
80	5CA1341.869-p6	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
81	5CA1341.869-p9	0	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
82	5CA1341.869-p11	0	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
83	5CA1341.871-p1	5	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
84	5CA1341.871-p3	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
85	5CA1341.871-p4	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
86	5CA1341.877-p5	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Gel#	DH lines/plants	Adult score	B5 (J11)		B8 (J12)		B7 (J13)		B6 (J14)		B2 (J15)		B4 (J16)		B1 (J17)		B3 (J18)	
			#503	#507	#508	#511	#515	#519	#520	#524	#528	#531	#533	#535	#541	#543	#544	#547
87	5CA1341.877-p11	5	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
88	5CA1341.877-p12	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
89	5CA1341.886-p1	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
90	5CA1341.886-p2	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
91	5CA1341.886-p3	5	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
92	5CA1341.886-p4	0	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
93	5CA1341.886-p11	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
94	5CA1341.886-p12	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
95	5CA1341.888-p2	0	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
96	5CA1341.888-p5	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
97	5CA1341.888-p6	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
98	5CA1341.888-p7	0	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-
99	5CA1341.888-p11	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
100	5CA1341.888-p7	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
101	5CA1341.888-p8	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
102	5CA1341.907-p8	0	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
103	5CA1341.920-p7	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
104	5CA1341.920-p8	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
105	5CA1341.597-p9	5	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+
106	5CA1341.597-p10	5	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+
107	5CA1341.608-p1	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
108	5CA1341.608-p4	0	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+
109	5CA1341.630-p11	5	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+
110	5CA1341.662-p1	2	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+
111	5CA1341.677-p7	0	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+
112	5CA1341.677-p8	0	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+
113	5CA1341.688-p3	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
114	5CA1341.688-p4	0	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+
SP1	*B. napus* cv. Polo	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SP2	*B. napus* cv. Westar	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
RP	*B. carinata*	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Evaluation of 31 of the 355 DH lines carrying different B genome chromosomes for resistance to PG4/PGt-type isolates #290CDN/#BL05-08RK

A total of 284 plants belonging to 31 DH lines were evaluated for resistance to 290CDN/BL05-08. These lines found to carry different B genome chromosomes. All six DH lines carrying the chromosome B3 and B7 showed greater resistance at adult plant stage (score <1.5) compared to the lines lacking these two chromosomes together. This is also evident from 15 of the 16 lines, which are carrying either B3 or B7 chromosomes showed high susceptibility (adult score >1.5) to these isolates (Table 7). Previously, we (Fredua-Agyemann et al. 2014) showed that the chromosome B3 carry resistance to a PG2-type isolate #3356. The results from this study show that B3 alone is not capable of conferring resistance to 290CDN/BL05-08; however, resistance gene of this chromosome along with the gene of B7 is capable of displaying resistance to 290CDN/BL05-08. This can also be seen from the single DH line carrying five chromosomes including B7 but lacking the chromosome B3 showed high susceptibility to these isolates. Seed set in most of the lines was poor – apparently due to presence of alien B chromosomes. Correlation between cotyledon and adult plant resistance in this population was 0.63 (Fig. 2).

Table 7. Evaluation of DH lines, derived from advanced backcross generation plants of (*Brassica napus* cv. Polo × *B. carinata* accession #98-14513) × *B. napus* cv. Westar (PCW), carrying different B genome chromosomes for resistance to 290CDN/BL05-08.

DH lines	Second test									First test		
	Cotyledon resistance			Adult plant resistance			Seed weights (g) / plant			n	Adult resis-tance	B genome content
	n	Range	Mean ± SE	n	Range	Mean ± SE	n	Range	Mean ± SE			
5CA1341.574	8	9-9	9.00 ±0.00	7	0-5	3.43 ±0.72	3	0.61-0.74	0.66 ±0.04	1	0	B7
5CA1341.577	8	3-9	8.25 ±0.75	8	5	5.00 ±0.00	4	0.00-0.97	0.26 ±0.24	1	1	B7
5CA1341.596	8	7-9	8.75 ±0.25	7	0-5	3.00 ±0.87	5	0.02-2.18	0.83 ±0.40	1	0	B1,2,6,7,8
5CA1341.597	8	3-9	6.50 ±0.91	5	0-1	0.20 ±0.20	2	0.03-0.15	0.09 ±0.06	2	5	B3,7
5CA1341.608	6	1-9	4.67 ±1.41	5	0-2	1.20 ±0.37	1	0.10-0.10	0.10 ±0.00	2	0	B3,7
5CA1341.612	8	3-9	7.50 ±0.82	8	1-4	2.25 ±0.37	8	0.06-1.60	0.74 ±0.20	1	1	No
5CA1341.621	8	1-9	6.00 ±0.93	2	0-1	2.00 ±1.00	4	0.08-3.89	1.57 ±0.91	1	1	B7
5CA1341.627	8	3-9	7.00 ±1.00	7	1-5	2.71 ±0.52	3	0.01-0.40	0.25 ±0.12	1	1	No
5CA1341.630	18	1-9	4.33 ±0.56	16	0-2	0.44 ±0.16	4	0.01-0.22	0.09 ±0.04	1	5	B3,7
5CA1341.662	2	3-5	4.00 ±1.00	2	0	0.00 ±0.00	1	0.14-0.14	0.14 ±0.00	1	2	B3,7
5CA1341.676	8	5-9	7.25 ±0.59	8	0-5	1.75 ±0.80	7	0.12-1.99	1.09 ±0.25	1	1	No
5CA1341.677	21	0-9	4.71 ±0.73	22	0-5	0.55 ±0.24	1	0.00-0.00	0.00 ±0.00	2	0	B3,7
5CA1341.681	8	9-9	9.00 ±0.00	4	5	5.00 ±0.00	0			1	1	B7
5CA1341.688	29	0-7	2.97 ±0.43	28	0-2	0.21 ±0.09	7	0.05-0.25	0.13 ±0.03	2	0	B3,7
5CA1341.702	12	0-9	6.58 ±0.87	9	0-5	1.67 ±0.53	8	0.01-1.50	0.72 ±0.21	1	1	B7
5CA1341.728	14	3-9	7.14 ±0.68	8	0-5	2.38 ±0.91	5	0.04-0.38	0.20 ±0.07	1	1	B7
5CA1341.745	4	0-0	0.00 ±0.00	3	0-2	1.00 ±0.58	0			1	3	B3
5CA1341.754	7	9-9	9.00 ±0.00	7	2-5	3.43 ±0.43	2	0.02-0.18	0.10 ±0.08	2	0	B7
5CA1341.759	8	5-9	8.00 ±0.53	6	1-5	3.67 ±0.61	1	0.09-0.09	0.09 ±0.00	4	0.25	B3
5CA1341.817	4	5-9	7.50 ±0.96	1	4	4.00 ±0.00	1	0.16-0.16	0.16 ±0.00	2	0	No
5CA1341.839	8	0-9	6.13 ±1.03	8	1-5	3.38 ±0.50	1	0.44-0.44	0.44 ±0.00	6	0	B7,8
5CA1341.843	8	1-9	7.00 ±1.00	8	2-5	3.25 ±0.31	1	0.37-0.37	0.37 ±0.00	2	0	B7
5CA1341.845	8	1-9	6.75 ±1.10	5	2-5	2.80 ±0.58	3	0.13-0.58	0.36 ±0.13	1	5	B7,8
5CA1341.857	8	1-9	6.25 ±1.00	5	0-4	2.60 ±0.75	1	0.21-0.21	0.21 ±0.00	3	3.33	B7
5CA1341.862	7	0-9	4.29 ±1.60	5	0-5	2.80 ±0.86	3	0.27-1.16	0.58 ±0.29	5	1	B7
5CA1341.863	8	5-9	8.50 ±0.50	7	2-5	4.00 ±0.44	0			4	1.33	B7
5CA1341.865	8	5-9	8.25 ±0.53	7	2-3	2.71 ±0.18	5	0.01-0.73	0.25 ±0.13	1	0	No
5CA1341.867	8	1-9	5.75 ±1.25	8	1-4	2.00 ±0.42	8	0.03-0.92	0.45 ±0.11	2	2.5	B7
5CA1341.868	8	3-9	7.00 ±0.85	7	1-5	3.86 ±0.55	0			8	0.8	B7,8
5CA1341.871	8	0-9	5.00 ±1.30	5	4-5	4.60 ±0.24	4	0.16-0.50	0.28 ±0.08	3	1.67	B7
5CA1341.886	8	3-9	7.25 ±0.80	7	0-1	1.71 ±0.29	4	0.01-0.57	0.22 ±0.13	6	0.83	B7
Total	**284**		**6.46 ±0.36**	**235**		**2.50 ±0.25**			**0.38 ±0.07**			

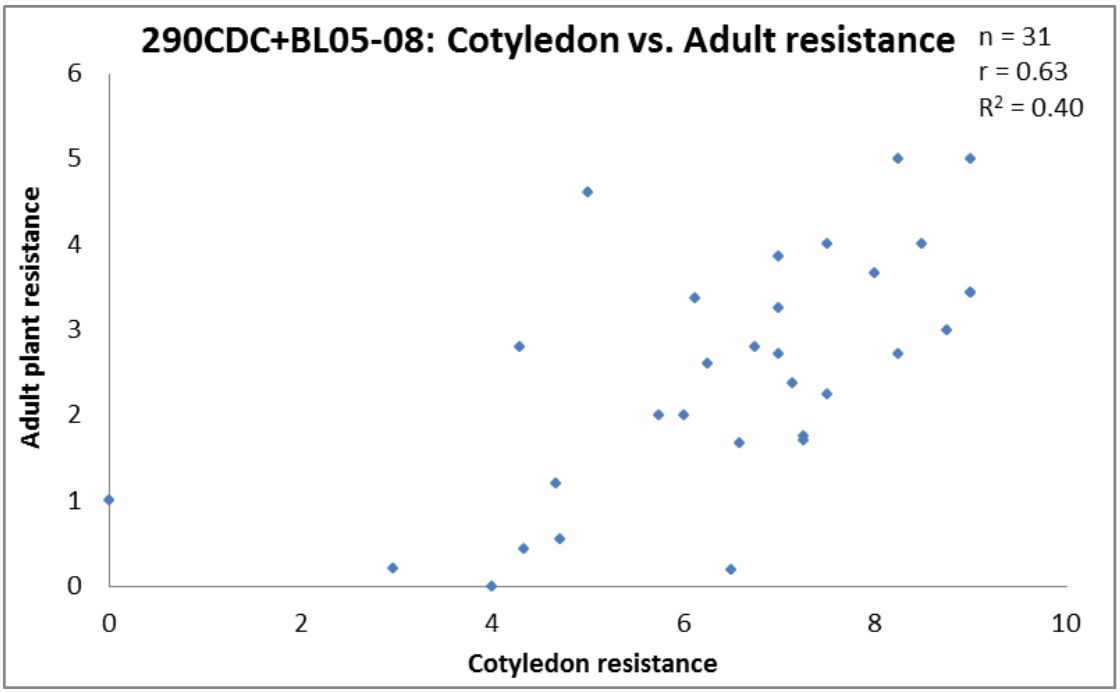

Fig. 2. Relationship between cotyledon and adult plant resistance to the *Leptosphaeria maculans* PG4/PGt-type isolates #290CDN/#BL05-08RK in the DH population derived from advanced backcross generation plants of (*Brassica napus* cv. Polo × *B. carinata* accession #98-14513) × *B. napus* cv. Westar (PCW) carrying different B genome chromosomes. *Same DH line tested for resistance to both isolates; mean data of the two isolates presented.*

Relationship between resistance to #290CDN and #BL05-08RK at cotyledon and adult plant stages

To test whether the two isolates, 290CDN and BL05-08RK, display similar resistance reaction, correlation between the two isolates was calculated for cotyledon and adult plant resistance (Fig. 3). Coefficient of correlation between these two isolates for both cotyledon and adult plant resistance was 0.61 indicating that the two isolates behaved similarly. Therefore, for simplicity, mean data of 290CDN/BL05-05RK from further study is presented in the following sections.

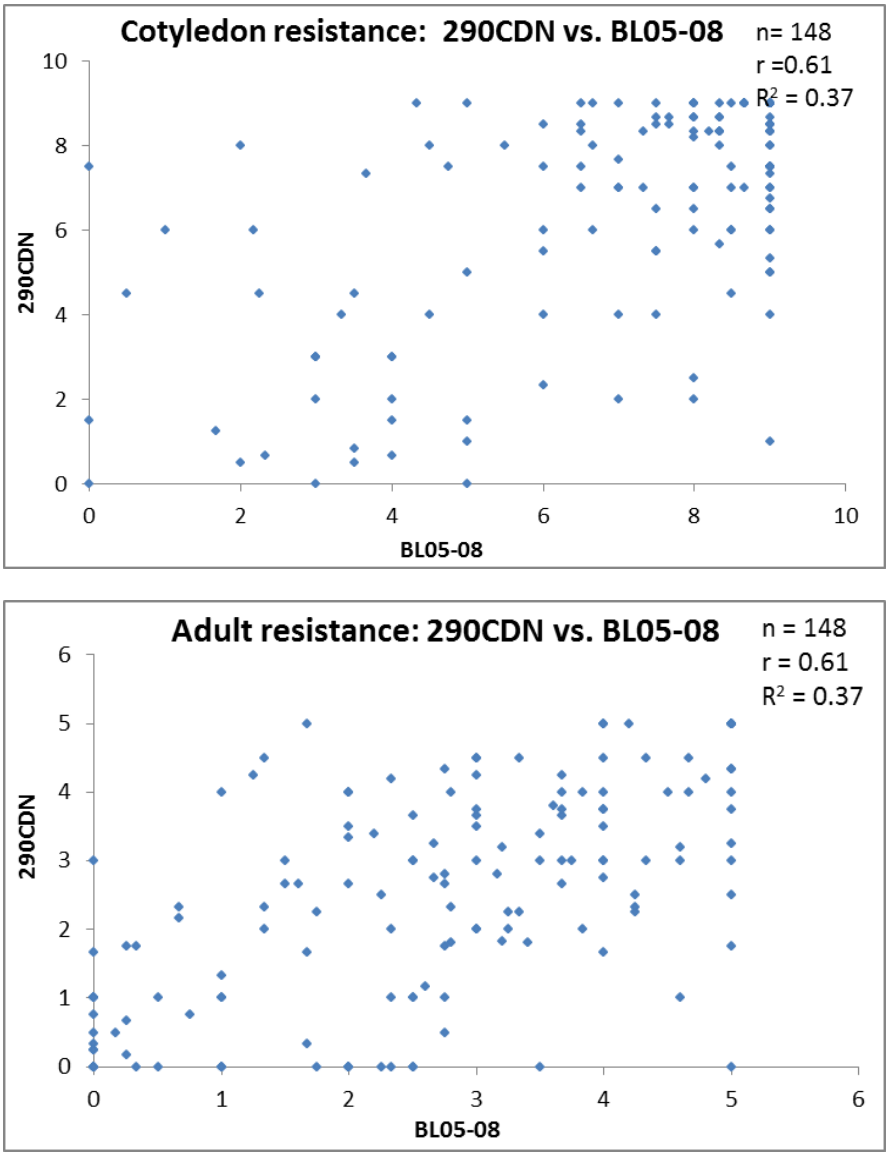

Fig. 3. Relationship between the *Leptosphaeria maculans* PG4-type isolate #290CDN and PGt-type isolate #BL05-08RK for cotyledon and adult plant resistance in the DH population derived from advanced backcross generation plants of (*Brassica napus* cv. Polo × *B. carinata* accession #98-14513) × *B. napus* cv. Westar (PCW). *Same DH line tested for resistance to both isolates*.

Evaluation of the additional 150 DH lines for resistance to PG4/PGt-type isolates #290CDN/#BL05-08-RK

In the second phase of testing, a total 1,385 plants belonging to 150 DH lines were evaluated for cotyledon resistance to 290CDN/BL05-08. Two lines could not be scored at adult plant stage; therefore, adult plant resistance data obtained from 148 lines. In this DH population, only four (2.6%) lines had disease score of ≤1.0 for cotyledon resistance − three of these showed good rsistance (disease score of ≤1.0) at adult plant stage (Table 8; Fig. 3). On the other hand, 40 DH lines had disease score of ≤1.0 for adult plant resistance despite these lines had disease score of ≥1 for cotyledon resistance (Table 8). Thus, 43 of the 148 DH lines, i.e. 29% of the lines, showed resistance to these isolates at adult plant stage. Mean seed set in the resistant lines was 0.48 g/plant (range = 0.00−1.72 g); this is similar to mean seed set in the whole DH population (mean = 0.48 ±0.04; range = 0.00−2.06 g/plant). The poor seed set in the DH population compared to seed set in the check cultivars Westar/Polo (2−4 g/plant) is apparently due to the presence of B genome chromosome(s). Scatter diagram of the 148 DH lines for cotyledon and adult plant resistance is presented in Fig. 3. Correlation between cotyledon and adult plant resistance was 0.65.

Table 8. Evaluation of 150 DH lines derived from advanced backcross generation plants of (*Brassica napus* cv. Polo × *B. carinata* accession #98-14513) × *B. napus* cv. Westar (PCW) for resistance to PG4/PGt-type isolates 290CDN/BL05-08.

DH lines	Cotyledon resistance*			Adult plant resistance*			Seed weight (g/plant)		
	N**	Range	Mean ± SE	N**	Range	Mean ± SE	N**	Range	Mean ± SE
5CA1341.572	13	0-9	2.85 ±0.76	12	0-2	0.25 ±0.18	1	0.17-0.17	0.17 ±0.00
5CA1341.573	12	9-9	9.00 ±0.00	9	1-5	2.78 ±0.43	6	0.15-0.98	0.61 ±0.15
5CA1341.576	8	9-9	9.00 ±0.00	4	2-5	4.25 ±0.75	4	0.01-0.92	0.46 ±0.19
5CA1341.579	12	5-9	8.50 ±0.36	10	1-5	2.90 ±0.55	8	0.02-0.84	0.43 ±0.10
5CA1341.580	7	5-9	8.14 ±0.59	6	0-5	2.00 ±0.97	5	0.13-1.54	0.62 ±0.25
5CA1341.582	8	3-9	5.00 ±0.76	8	0-1	0.13 ±0.13	0		
5CA1341.583	12	3-9	7.83 ±0.58	11	1-5	4.00 ±0.49	6	0.18-1.01	0.41 ±0.12
5CA1341.584	6	5-9	8.00 ±0.68	4	3-5	3.50 ±0.50	2	0.01-0.99	0.50 ±0.49
5CA1341.585	12	7-9	8.33 ±0.28	12	0-5	3.25 ±0.51	9	0.02-1.88	0.57 ±0.20
5CA1341.590	3	0-0	0.00 ±0.00	3	0-2	0.67 ±0.67	0		
5CA1341.591	12	5-9	8.33 ±0.38	11	0-5	2.55 ±0.41	10	0.01-1.46	0.47 ±0.17
5CA1341.593	4	5-7	6.00 ±0.58	3	0-5	1.67 ±1.67	0		
5CA1341.595	12	7-9	8.67 ±0.22	10	0-5	3.50 ±0.65	9	0.00-0.64	0.15 ±0.07
5CA1341.598	15	3-9	6.20 ±0.64	13	0-5	0.77 ±0.47	8	0.01-0.20	0.09 ±0.03

DH lines	Cotyledon resistance*			Adult plant resistance*			Seed weight (g/plant)		
	N**	Range	Mean ± SE	N**	Range	Mean ± SE	N**	Range	Mean ± SE
5CA1341.600	8	5-9	7.75 ±0.53	7	0-5	2.00 ±0.87	7	0.00-2.89	0.91 ±0.41
5CA1341.601	2	3-5	4.00 ±1.00	2	0-0	0.00 ±0.00	0		
5CA1341.603	3	3-5	3.67 ±0.67	3	0-0	0.00 ±0.00	0		
5CA1341.604	20	3-9	6.60 ±0.47	19	0-5	0.79 ±0.38	6	0.04-0.18	0.11 ±0.02
5CA1341.605	6	3-9	6.33 ±0.84	6	0-3	1.33 ±0.49	1	0.22-0.22	0.22 ±0.00
5CA1341.606	8	5-9	8.25 ±0.53	6	0-3	1.17 ±0.54	5	0.21-1.60	0.80 ±0.25
5CA1341.611	15	1-9	5.40 ±0.68	13	0-5	1.46 ±0.53	0		
5CA1341.615	25	1-9	5.32 ±0.53	21	0-5	0.71 ±0.30	4	0.07-2.30	0.74 ±0.53
5CA1341.616	6	0-9	4.17 ±1.60	6	0-5	1.00 ±0.82	0		
5CA1341.618	12	5-9	8.33 ±0.45	11	1-5	2.36 ±0.41	8	0.03-2.72	1.33 ±0.32
5CA1341.619	1	1-1	1.00 ±0.00	1	1-1	1.00 ±0.00	0		
5CA1341.623	12	9-9	9.00 ±0.00	9	1-5	2.89 ±0.59	7	0.00-1.61	0.59 ±0.20
5CA1341.624	8	3-9	6.50 ±0.98	8	0-5	2.75 ±0.67	8	0.15-2.32	0.89 ±0.27
5CA1341.625	8	3-9	7.25 ±0.80	8	2-5	3.50 ±0.33	8	0.01-2.60	0.64 ±0.30
5CA1341.626	12	5-9	8.17 ±0.39	10	2-5	3.70 ±0.40	8	0.12-1.84	0.44 ±0.21
5CA1341.629	8	7-9	8.25 ±0.37	8	0-5	1.63 ±0.63	7	0.01-2.31	1.32 ±0.30
5CA1341.631	12	9-9	9.00 ±0.00	10	1-5	2.30 ±0.60	8	0.00-1.68	0.49 ±0.20
5CA1341.633	8	0-9	7.13 ±1.08	8	0-5	3.50 ±0.71	5	0.00-1.80	0.43 ±0.35
5CA1341.635	5	0-9	4.00 ±1.48	5	0-1	0.20 ±0.20	1	0.08-0.08	0.08 ±0.00
5CA1341.636	8	3-9	5.50 ±0.73	8	0-3	0.50 ±0.38	5	0.12-0.39	0.22 ±0.05
5CA1341.637	6	0-9	3.83 ±1.68	5	0-5	1.40 ±0.93	0		
5CA1341.638	8	5-9	8.25 ±0.53	8	3-5	3.88 ±0.35	4	0.22-1.00	0.56 ±0.16
5CA1341.639	8	1-9	8.00 ±1.00	7	3-5	4.71 ±0.29	2	0.12-0.65	0.39 ±0.27
5CA1341.641	8	3-9	6.75 ±0.80	8	0-5	3.75 ±0.82	6	0.00-0.36	0.10 ±0.06
5CA1341.643	8	3-9	7.75 ±0.84	8	3-5	4.25 ±0.37	3	0.10-0.21	0.17 ±0.04
5CA1341.645	12	3-9	8.17 ±0.52	11	1-5	3.00 ±0.43	6	0.01-0.89	0.42 ±0.17
5CA1341.647	11	9-9	9.00 ±0.00	6	0-5	2.33 ±0.71	3	0.22-1.30	0.83 ±0.32
5CA1341.649	12	7-9	8.83 ±0.17	10	1-5	3.90 ±0.53	6	0.01-1.39	0.40 ±0.24
5CA1341.650	8	3-9	7.75 ±0.84	3	5-5	5.00 ±0.00	3	0.06-0.48	0.23 ±0.13
5CA1341.653	8	9-9	9.00 ±0.00	7	1-5	3.86 ±0.59	6	0.00-0.38	0.13 ±0.05
5CA1341.655	10	0-7	3.70 ±0.79	10	0-1	0.10 ±0.10	3	0.00-0.37	0.13 ±0.12
5CA1341.657	12	3-9	7.33 ±0.54	11	0-5	1.82 ±0.42	9	0.00-2.08	0.70 ±0.23
5CA1341.658	3	1-9	5.00 ±2.31	2	0-0	0.00 ±0.00	2	0.80-1.20	1.00 ±0.20
5CA1341.659	7	9-9	9.00 ±0.00	0			0		
5CA1341.660	12	5-9	8.67 ±0.33	11	0-5	3.00 ±0.54	6	0.03-0.38	0.21 ±0.06
5CA1341.661	7	0-9	4.00 ±1.60	7	0-4	1.43 ±0.61	1	0.02-0.02	0.02 ±0.00
5CA1341.663	5	1-5	3.00 ±0.63	5	0-1	0.60 ±0.24	3	0.01-0.26	0.11 ±0.08
5CA1341.664	1	3-3	3.00 ±0.00	1	0-0	0.00 ±0.00	0		
5CA1341.665	16	0-9	4.44 ±0.62	15	0-1	0.27 ±0.12	1	0.01-0.01	0.01 ±0.00

DH lines	Cotyledon resistance*			Adult plant resistance*			Seed weight (g/plant)		
	N**	Range	Mean ± SE	N**	Range	Mean ± SE	N**	Range	Mean ± SE
5CA1341.666	7	3-9	6.43 ±0.72	8	0-2	0.25 ±0.25	1	0.28-0.28	0.28 ±0.00
5CA1341.669	12	5-9	8.17 ±0.46	4	2-3	2.25 ±0.25	1	0.11-0.11	0.11 ±0.00
5CA1341.672	25	0-9	5.72 ±0.62	22	0-3	0.27 ±0.15	8	0.00-0.54	0.16 ±0.06
5CA1341.673	8	1-9	5.25 ±1.10	8	0-5	1.25 ±0.62	7	0.01-1.67	0.97 ±0.20
5CA1341.678	12	9-9	9.00 ±0.00	10	0-5	2.80 ±0.47	7	0.02-1.56	0.81 ±0.21
5CA1341.679	8	3-9	7.00 ±0.85	8	0-5	2.75 ±0.59	5	0.00-1.50	0.59 ±0.29
5CA1341.680	12	5-9	8.17 ±0.46	10	1-5	3.20 ±0.47	4	0.29-1.42	0.71 ±0.25
5CA1341.682	8	3-9	6.75 ±0.96	7	3-5	4.43 ±0.37	5	0.27-0.60	0.44 ±0.05
5CA1341.684	7	0-9	3.29 ±1.57	3	2-5	3.67 ±0.88	4	0.01-0.57	0.28 ±0.12
5CA1341.686	8	5-9	8.25 ±0.53	8	0-5	2.63 ±0.68	6	0.28-1.17	0.63 ±0.14
5CA1341.689	8	5-9	7.75 ±0.65	8	1-5	3.38 ±0.63	5	0.00-0.47	0.31 ±0.08
5CA1341.691	16	0-7	2.69 ±0.71	14	0-4	0.71 ±0.30	7	0.01-0.28	0.08 ±0.04
5CA1341.692	12	2-9	7.83 ±0.79	9	0-5	1.78 ±0.55	10	0.29-2.62	0.97 ±0.22
5CA1341.695	16	0-9	5.63 ±0.96	10	0-5	1.50 ±0.50	11	0.06-2.42	0.84 ±0.19
5CA1341.696	5	0-7	3.60 ±1.17	5	0-0	0.00 ±0.00	0		
5CA1341.698	2	5-9	7.00 ±2.00	0			0		
5CA1341.699	8	0-9	7.88 ±1.13	8	0-5	3.25 ±0.75	3	0.04-0.48	0.23 ±0.13
5CA1341.703	8	3-9	8.25 ±0.75	8	0-5	2.50 ±0.76	6	0.06-1.90	0.82 ±0.26
5CA1341.704	32	0-9	3.72 ±0.59	30	0-3	0.40 ±0.16	1	0.05-0.05	0.05 ±0.00
5CA1341.705	4	0-9	2.50 ±2.18	4	0-0	0.00 ±0.00	0		
5CA1341.706	3	3-7	4.33 ±1.33	3	0-0	0.00 ±0.00	2	0.02-0.13	0.08 ±0.06
5CA1341.707	6	1-9	6.67 ±1.50	6	0-5	3.33 ±0.92	4	0.00-0.19	0.08 ±0.05
5CA1341.708	8	1-9	6.50 ±1.05	2	0-5	2.50 ±2.50	3	0.11-2.52	1.27 ±0.70
5CA1341.711	12	9-9	9.00 ±0.00	11	0-4	1.73 ±0.41	10	0.00-1.76	0.41 ±0.17
5CA1341.713	1	1-1	1.00 ±0.00	1	5-5	5.00 ±0.00	1	2.06-2.06	2.06 ±0.00
5CA1341.714	12	7-9	8.50 ±0.26	11	1-5	3.00 ±0.50	9	0.01-1.91	0.52 ±0.19
5CA1341.715	31	1-9	6.94 ±0.53	12	0-5	2.33 ±0.66	7	0.90-2.47	1.52 ±0.21
5CA1341.717	12	5-9	8.50 ±0.36	11	0-5	2.18 ±0.52	9	0.01-1.40	0.47 ±0.15
5CA1341.718	16	0-9	5.69 ±0.90	12	0-5	2.58 ±0.61	8	0.06-2.88	1.16 ±0.46
5CA1341.720	11	3-9	8.09 ±0.62	7	1-5	2.71 ±0.61	4	0.00-0.82	0.30 ±0.18
5CA1341.721	22	1-9	7.00 ±0.59	13	0-5	2.15 ±0.56	16	0.00-2.59	0.86 ±0.19
5CA1341.724	47	0-9	5.83 ±0.45	33	0-5	3.03 ±0.30	20	0.02-2.08	0.53 ±0.13
5CA1341.729	12	0-7	2.25 ±0.63	10	0-1	0.20 ±0.13	0		
5CA1341.732	7	0-3	1.43 ±0.43	7	0-5	1.14 ±0.70	1	0.08-0.08	0.08 ±0.00
5CA1341.733	12	9-9	9.00 ±0.00	11	0-5	3.18 ±0.52	6	0.02-1.19	0.68 ±0.16
5CA1341.734	12	7-9	8.83 ±0.17	10	0-5	2.20 ±0.66	6	0.03-1.00	0.51 ±0.15
5CA1341.736	12	5-9	8.33 ±0.45	12	0-5	1.67 ±0.54	11	0.00-1.28	0.44 ±0.13
5CA1341.738	4	9-9	9.00 ±0.00	4	2-5	3.50 ±0.65	4	0.04-0.92	0.29 ±0.21

DH lines	Cotyledon resistance*			Adult plant resistance*			Seed weight (g/plant)		
	N**	Range	Mean ± SE	N**	Range	Mean ± SE	N**	Range	Mean ± SE
5CA1341.741	1	3-3	3.00 ±0.00	1	1-1	1.00 ±0.00	0		
5CA1341.750	12	0-9	4.08 ±0.75	12	0-4	1.42 ±0.42	1	0.60-0.60	0.60 ±0.00
5CA1341.751	2	3-3	3.00 ±0.00	2	0-0	0.00 ±0.00	0		
5CA1341.753	10	3-9	7.60 ±0.73	10	0-5	2.70 ±0.65	7	0.01-2.30	0.66 ±0.30
5CA1341.757	6	1-9	5.33 ±1.41	4	0-1	0.75 ±0.25	4	1.08-2.43	1.72 ±0.36
5CA1341.760	12	5-9	7.83 ±0.46	10	0-5	2.60 ±0.70	4	0.00-0.21	0.07 ±0.05
5CA1341.761	3	3-5	3.67 ±0.67	3	0-5	2.67 ±1.45	0		
5CA1341.763	8	3-9	6.50 ±1.05	5	2-5	3.40 ±0.60	0		
5CA1341.765	5	0-5	3.00 ±1.22	4	0-5	1.75 ±1.18	0		
5CA1341.767	8	1-9	5.75 ±1.06	8	0-3	0.50 ±0.38	0		
5CA1341.769	5	0-7	2.00 ±1.26	5	0-0	0.00 ±0.00	0		
5CA1341.773	12	1-9	6.33 ±0.90	11	1-5	3.73 ±0.54	3	0.00-0.14	0.06 ±0.04
5CA1341.775	5	3-9	6.60 ±1.17	5	1-5	2.40 ±0.75	0		
5CA1341.776	12	7-9	8.83 ±0.17	10	1-5	3.70 ±0.42	9	0.00-1.30	0.38 ±0.14
5CA1341.779	2	0-9	4.50 ±4.50	2	0-0	0.00 ±0.00	0		
5CA1341.780	2	5-9	7.00 ±2.00	2	4-5	4.50 ±0.50	0		
5CA1341.782	12	5-9	8.50 ±0.36	12	1-5	3.92 ±0.38	6	0.03-0.19	0.12 ±0.03
5CA1341.784	2	7-7	7.00 ±0.00	1	5-5	5.00 ±0.00	0		
5CA1341.786	12	7-9	8.83 ±0.17	12	2-5	3.50 ±0.38	7	0.11-1.65	0.63 ±0.19
5CA1341.788	3	1-9	4.33 ±2.40	2	1-1	1.00 ±0.00	0		
5CA1341.791	4	9-9	9.00 ±0.00	4	3-5	4.50 ±0.50	2	0.52-0.98	0.75 ±0.23
5CA1341.800	12	7-9	8.83 ±0.17	12	0-5	3.17 ±0.53	10	0.00-1.55	0.52 ±0.18
5CA1341.804	4	1-9	5.00 ±1.83	3	0-2	0.67 ±0.67	0		
5CA1341.805	8	3-9	7.50 ±0.82	8	3-5	4.25 ±0.37	2	0.07-0.22	0.15 ±0.08
5CA1341.807	12	5-9	8.33 ±0.38	8	2-5	4.50 ±0.38	3	0.01-0.42	0.17 ±0.13
5CA1341.811	1	5-5	5.00 ±0.00	1	1-1	1.00 ±0.00	0		
5CA1341.812	4	0-5	2.75 ±1.03	4	0-2	1.00 ±0.58	2	0.00-0.21	0.11 ±0.10
5CA1341.814	12	7-9	8.67 ±0.22	5	0-5	2.60 ±1.08	0		
5CA1341.818	12	9-9	9.00 ±0.00	11	1-5	3.45 ±0.59	7	0.02-1.02	0.47 ±0.15
5CA1341.819	4	1-5	3.00 ±0.82	4	0-1	0.50 ±0.29	0		
5CA1341.820	12	7-9	8.83 ±0.17	10	3-5	4.50 ±0.27	1	1.20-1.20	1.20 ±0.00
5CA1341.821	12	0-9	8.08 ±0.75	7	0-5	3.57 ±0.75	4	0.01-0.07	0.03 ±0.01
5CA1341.823	1	3-3	3.00 ±0.00	1	0-0	0.00 ±0.00	0		
5CA1341.824	8	7-9	8.50 ±0.33	5	4-5	4.60 ±0.24	0		
5CA1341.825	5	0-9	4.80 ±1.56	4	0-5	3.50 ±1.19	0		
5CA1341.826	8	7-9	8.25 ±0.37	4	5-5	5.00 ±0.00	0		
5CA1341.828	8	9-9	9.00 ±0.00	5	5-5	5.00 ±0.00	3	0.12-0.20	0.17 ±0.03
5CA1341.829	8	7-9	8.75 ±0.25	4	5-5	5.00 ±0.00	2	0.76-1.26	1.01 ±0.25

DH lines	Cotyledon resistance*			Adult plant resistance*			Seed weight (g/plant)		
	N**	Range	Mean ± SE	N**	Range	Mean ± SE	N**	Range	Mean ± SE
5CA1341.833	2	0-3	1.50 ±1.50	2	0-2	1.00 ±1.00	0		
5CA1341.836	3	0-3	1.00 ±1.00	3	0-0	0.00 ±0.00	0		
5CA1341.840	12	3-9	7.33 ±0.59	5	3-5	4.40 ±0.40	4	0.00-0.16	0.05 ±0.04
5CA1341.844	12	1-9	5.50 ±0.78	10	1-5	3.90 ±0.46	5	0.01-0.23	0.14 ±0.04
5CA1341.848	8	3-9	4.00 ±0.76	8	0-5	1.88 ±0.83	0		
5CA1341.852	8	3-9	6.50 ±0.91	8	1-5	3.75 ±0.45	1	0.00-0.00	0.00 ±0.00
5CA1341.853	12	5-9	7.17 ±0.52	11	0-5	2.64 ±0.59	4	0.00-0.53	0.27 ±0.12
5CA1341.855	11	7-9	8.27 ±0.30	8	2-5	3.25 ±0.45	6	0.02-0.48	0.17 ±0.07
5CA1341.861	3	7-9	7.67 ±0.67	2	3-5	4.00 ±1.00	2	0.03-0.09	0.06 ±0.03
5CA1341.864	12	9-9	9.00 ±0.00	11	0-5	2.45 ±0.47	9	0.01-1.83	0.68 ±0.23
5CA1341.874	8	3-9	8.00 ±0.76	8	1-4	2.50 ±0.33	2	0.05-0.61	0.33 ±0.28
5CA1341.881	5	3-9	7.40 ±1.17	2	0-3	1.50 ±1.50	0		
5CA1341.898	12	9-9	9.00 ±0.00	12	1-5	1.83 ±0.44	9	0.14-1.91	0.87 ±0.21
5CA1341.909	7	7-9	8.43 ±0.37	6	0-4	1.67 ±0.67	6	0.34-1.56	0.71 ±0.19
5CA1341.911	12	9-9	9.00 ±0.00	3	0-5	3.00 ±1.53	2	0.03-0.52	0.28 ±0.25
5CA1341.914	12	0-9	3.25 ±0.93	10	0-5	1.50 ±0.76	0		
5CA1341.915	3	1-7	5.00 ±2.00	3	0-1	0.33 ±0.33	0		
5CA1341.916	8	0-9	3.38 ±0.96	8	0-2	0.38 ±0.26	0		
5CA1341.917	12	0-5	1.50 ±0.42	12	0-1	0.33 ±0.14	1	0.00-0.00	0.00 ±0.00
5CA1341.918	4	5-9	7.00 ±1.15	3	0-1	0.67 ±0.33	0		
Total	1,385 (150)	0-9	6.38 ±0.19	1,141 (150)	0-5	2.25 ±0.12	564 (107)	0.00-2.06	0.48 ±0.04

*Cotyledon resistance in 0 - 9 scale and adult plant resistance in 1 – 5 scale, where 0 = highly resistant (≈ *B. carinata*).
**'Zero' indicates that the plant(s) died.

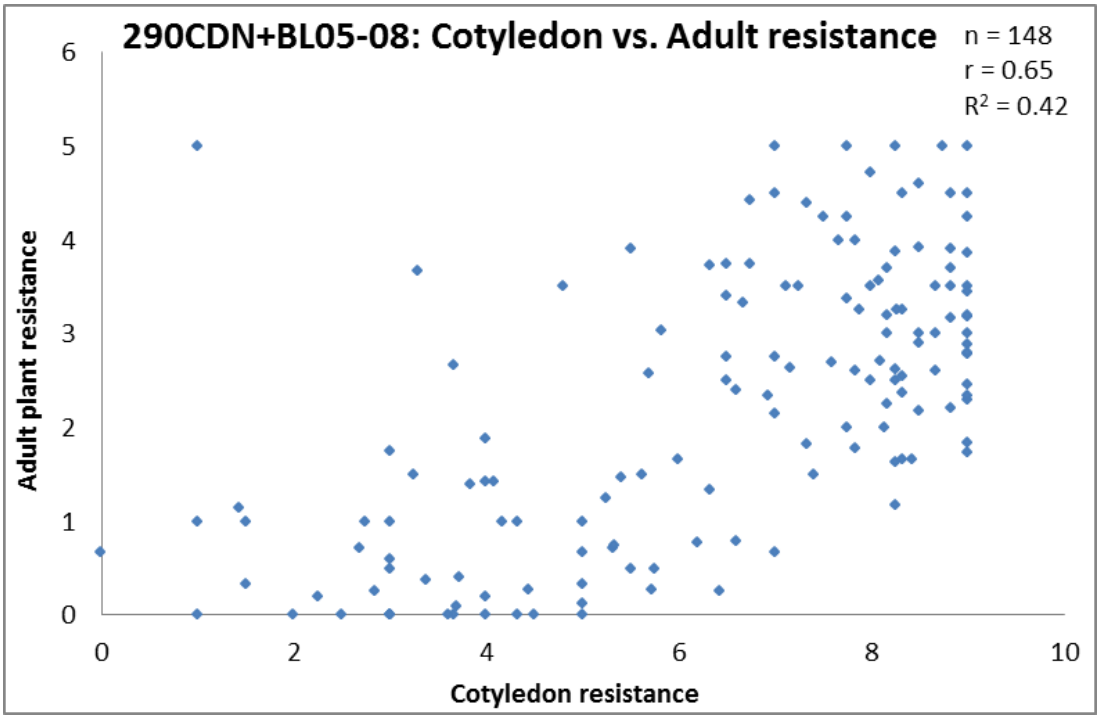

Fig. 3. Relationship between cotyledon and adult plant resistance to *Leptosphaeria maculans* PG4/PGt-type isolates #290CDN/#BL05-08RK in the DH population derived from advanced backcross generation plants of (*Brassica napus* cv. Polo × *B. carinata* accession #98-14513) × *B. napus* cv. Westar (PCW).

Evaluation of the additional 37 pedigree families for resistance to PG4/PGt-type isolates #290CDN/#BL05-08-RK

In the second phase of testing, a total of 358 seedlings belonging to 37 families were evaluated for adult-plant resistance to 290CDN/BL05-08 where 35% (13/37) families had disease score ≤1.0 (Table 9) indicating that resistance to these isolates is present in this population. However, seed set in all plants was poor (mean = 0.04 ±0.01) – apparently due to the presence of B genome chromosomes. Therefore, self-pollination will be needed for introgression of resistance from the B genome chromosome to the *B. napus* AC genome chromosome(s).

Table 9. Adult plant responses to *L. maculans* PG4/PGt-type isolates #290CDN/ #BL05-08RK in 37 pedigree lines derived from (*B. carinata* accession #98-14513 × *Brassica napus* cv. Westar) × *B. napus* cv. Westar (CWW) and (*Brassica napus* cv. Polo × *B. carinata* accession #98-14513) × *B. napus* cv. Westar (PCW) interspecific crosses.

Pedigree			Adult plant resistance			Seed weight (g/plant)		
Cross	Gen.	Family	N	Range	Mean ± SE	N*	Range	Mean ± SE
CWW	BC_3S_2	5CA1250.322	4	0-0	0.00 ±0.00	0		
CWW	BC_3S_2	5CA1250.325	6	0-5	2.67 ±0.95	2	0.01-0.01	0.01 ±0.00
CWW	BC_2S_3	5CA1250.362	4	0-2	0.50 ±0.50	0		
CWW	BC_2S_3	5CA1250.364	4	1-5	2.25 ±0.48	1	0.01-0.01	0.01
CWW	BC_2S_6	5CA1250.401	8	1-5	3.50 ±0.60	5	0.01-0.21	0.11 ±0.04
CWW	BC_2S_6	5CA1250.598	6	0-3	1.50 ±0.56	1	0.01-0.01	0.01
PCW	BC_3S_1	5CA1341.552	8	0-1	0.25 ±0.16	3	0.01-0.01	0.01 ±0.00
PCW	BC_3S_1	5CA1341.553	10	0-5	1.30 ±0.47	2	0.01-0.01	0.01 ±0.00
PCW	BC_3S_1	5CA1341.554	7	0-5	1.29 ±0.68	0		
PCW	BC_3S_1	5CA1341.555	14	0-5	2.00 ±0.50	6	0.00-0.11	0.06 ±0.02
PCW	BC_3S_1	5CA1341.556	11	0-3	0.73 ±0.27	1	0.01-0.01	0.01
PCW	BC_3S_1	5CA1341.557	7	0-2	0.43 ±0.30	0		
PCW	BC_3S_1	5CA1341.559	14	0-5	2.07 ±0.51	6	0.07-0.34	0.21 ±0.04
PCW	BC_3S_1	5CA1341.560	16	0-2	0.63 ±0.18	5	0.01-0.42	0.12 ±0.08
PCW	BC_3S_1	5CA1341.561	16	0-5	1.69 ±0.36	5	0.01-0.05	0.02 ±0.01
PCW	BC_3S_1	5CA1341.562	16	0-3	0.81 ±0.21	7	0.01-0.13	0.04 ±0.02
PCW	BC_3S_1	5CA1341.563	9	0-2	0.78 ±0.22	4	0.01-0.08	0.03 ±0.02
PCW	BC_3S_1	5CA1341.564	16	0-5	1.75 ±0.45	8	0.01-0.11	0.03 ±0.02
PCW	BC_3S_1	5CA1341.565	8	0-2	0.38 ±0.26	0		
PCW	BC_3S_1	5CA1341.566	14	1-5	3.00 ±0.46	4	0.01-0.03	0.02 ±0.00
PCW	BC_3S_1	5CA1341.567	16	0-5	1.56 ±0.46	4	0.01-0.16	0.05 ±0.04
PCW	BC_3S_1	5CA1341.568	14	0-5	1.57 ±0.48	3	0.01-0.18	0.09 ±0.05
PCW	BC_3S_1	5CA1341.569	8	0-4	1.25 ±0.56	0		
PCW	BC_3S_2	5CA1341.925	8	0-5	1.63 ±0.63	3	0.01-0.02	0.02 ±0.00
PCW	BC_3S_2	5CA1341.943	8	0-5	1.75 ±0.65	0		
PCW	BC_3S_2	5CA1341.945	4	0-2	0.50 ±0.50	0		
PCW	BC_3S_2	5CA1341.953	8	0-2	0.63 ±0.32	3	0.01-0.12	0.07 ±0.03
PCW	BC_3S_2	5CA1341.956	8	0-3	0.75 ±0.49	2	0.01-0.01	0.01 ±0.00
PCW	BC_3S_2	5CA1341.964	13	0-5	1.85 ±0.48	8	0.01-0.17	0.05 ±0.02
PCW	BC_3S_2	5CA1341.967	7	0-3	1.00 ±0.44	2	0.02-0.04	0.03 ±0.01
PCW	BC_3S_2	5CA1341.969	8	0-5	1.88 ±0.55	6	0.01-0.09	0.04 ±0.01
PCW	BC_3S_2	5CA1341.970	7	0-5	1.14 ±0.77	1	0.01-0.01	0.01
PCW	BC_3S_2	5CA1341.971	7	2-5	3.43 ±0.48	2	0.01-0.01	0.01 ±0.00
PCW	BC_3S_2	5CA1341.972	3	0-4	1.33 ±1.33	1	0.01-0.01	0.01
PCW	BC_3S_2	5CA1341.973	18	0-5	2.44 ±0.47	3	0.01-0.39	0.14 ±0.13
PCW	BC_3S_2	5CA1341.974	16	0-3	1.13 ±0.29	2	0.01-0.01	0.01 ±0.00
PCW	BC_3S_2	5CA1341.976	7	0-1	0.29 ±0.18	2	0.01-0.01	0.01 ±0.00
Total			**358 (37)**		**1.40 ±0.14**	**102 (29)**		**0.04 ±0.01**

*'Zero' indicates that the plant(s) died.

Evaluation of the DH lines for resistance to PG2-type isolate #3356

To diversify the PG2-type resistance in canola, the DH lines which are susceptible to PG4/PGt-type isolates 290CDN/BL05-08 were tested for resistance to a PG2-type isolate #3356 for the development of resistant lines carrying genome content introgressed from the B genome of *B. carinata*.

A total of 822 plants belonging to 127 DH lines were evaluated for resistance #3356 where majority of the 127 DH lines were susceptible to #3356 at both cotyledon and adult plants stage: Six (4.7%) of these lines had disease score of ≤1.0 for cotyledon resistance, however, five (3.9%) of these were resistant at adult plant stage (disease score of ≤1.0) (Table 10; Fig. 4). Considering the whole DH population of 127 DH lines, 24 lines died before maturity; therefore, adult-plant resistance could be scored for 103 lines. Of the 103 DH lines, 24 (23%) had disease score of ≤1.0 suggesting that adult-plant resistance to this isolate can be found in this population. Scatter diagram of the 103 DH lines for cotyledon and adult plant resistances is presented in Fig. 4. Correlation between cotyledon and adult plant resistance to #3356 was weak (r = 0.29); this is in contrast to the relationship between these two traits (cotyledon and adult plant resistance) found in case of resistance to 290CDN/BL05-08RK (r > 0.60) (Fig. 2, 3). However, few lines showing high resistance at cotyledon stage also showed high resistance at adult plant stage (Table 10, Fig. 4). Of the 454 plants belonging to 103 DH lines, 77% (351) produced seed. Mean seed set in this population was 0.64 ± 0.05 g/plant; however, some of the plants produced more than 2.0 g seed/plant.

Table 10. Evaluation of the DH lines, derived from advanced backcross generation plants of (*Brassica napus* cv. Polo × *B. carinata* accession #98-14513) × *B. napus* cv. Westar (PCW), and susceptible to PG4/PGt-type isolates 290CDN/BL05-08 for resistance to PG2-type isolate #3356.

DH lines	Cotyledon resistance*			Adult plant resistance*			Seed weight (g/plant)		
	N**	Range	Mean ± SE	N**	Range	Mean ± SE	N**	Range	Mean ± SE
5CA1341.572	8	1-7	4.25 ±0.92	5	0-1	0.40 ±0.24	0		
5CA1341.573	6	7-9	8.33 ±0.42	0			5	0.01-0.95	0.27± 0.18
5CA1341.574	6	9-9	9.00 ±0.00	0			6	0.09-0.60	0.28± 0.08
5CA1341.576	5	3-9	7.80 ±1.20	3	2-5	3.00 ±1.00	3	0.20-1.49	0.67± 0.41
5CA1341.577	6	1-9	6.00 ±1.34	4	0-2	1.25 ±0.48	2	0.44-1.91	1.18± 0.74
5CA1341.579	6	3-9	5.00 ±1.03	5	1-5	2.40 ±0.75	4	0.35-1.55	0.76± 0.27
5CA1341.580	6	9-9	9.00 ±0.00	0			6	0.00-0.95	0.30± 0.14
5CA1341.581	6	0-7	3.50 ±1.20	6	0-2	0.33 ±0.33	0		
5CA1341.583	6	7-9	8.67 ±0.33	5	2-5	3.60 ±0.60	5	0.17-1.38	0.60± 0.21
5CA1341.584	6	3-9	7.00 ±1.26	2	5-5	5.00 ±0.00	5	0.48-1.55	0.80± 0.19
5CA1341.585	6	9-9	9.00 ±0.00	2	5-5	5.00 ±0.00	5	0.01-1.63	0.77± 0.31
5CA1341.587	6	0-1	0.50 ±0.22	6	0-2	1.00 ±0.37	0		
5CA1341.589	6	0-9	6.00 ±1.90	1	5-5	5.00	2	0.07-0.26	0.17± 0.10
5CA1341.591	6	9-9	9.00 ±0.00	0			6	0.16-3.33	1.12± 0.52
5CA1341.592	6	0-1	0.33 ±0.21	4	0-2	0.50 ±0.50	5	0.36-4.31	1.73± 0.68
5CA1341.595	6	3-5	3.67 ±0.42	6	1-5	3.33 ±0.61	5	0.11-0.25	0.20± 0.02
5CA1341.596	6	7-9	8.33 ±0.42	0			3	0.05-0.51	0.25± 0.14
5CA1341.598	5	3-9	7.40 ±1.17	4	0-2	0.50 ±0.50	1	0.08-0.08	0.08±0.00
5CA1341.600	6	3-9	6.67 ±0.95	2	1-1	1.00 ±0.00	5	0.54-2.39	1.55± 0.34
5CA1341.604	9	3-9	5.44 ±0.80	8	0-1	0.25 ±0.16	1	0.13-0.13	0.13±0.00
5CA1341.605	5	5-9	7.40 ±0.75	5	0-0	0.00 ±0.00	0		
5CA1341.606	6	9-9	9.00 ±0.00	0			4	0.10-0.74	0.36± 0.14
5CA1341.611	6	7-9	8.33 ±0.42	6	0-1	0.50 ±0.22	0		
5CA1341.612	6	3-9	7.00 ±1.03	0			2	0.14-1.11	0.63± 0.49
5CA1341.615	9	3-9	7.00 ±0.67	7	0-5	1.29 ±0.75	3	0.01-1.01	0.54± 0.29
5CA1341.618	6	9-9	9.00 ±0.00	0			5	0.03-1.43	0.53± 0.27
5CA1341.621	10	7-9	8.40 ±0.31	3	3-3	3.00 ±0.00	7	0.00-2.09	1.09± 0.27
5CA1341.622	5	1-9	4.60 ±1.33	0			4	0.02-2.03	1.04± 0.41
5CA1341.623	6	3-7	4.33 ±0.67	5	4-5	4.60 ±0.24	4	0.00-0.62	0.31± 0.15
5CA1341.624	6	9-9	9.00 ±0.00	2	4-5	4.50 ±0.50	6	0.26-1.83	0.63± 0.25
5CA1341.625	6	5-9	6.67 ±0.80	6	3-5	3.83 ±0.40	2	0.00-0.44	0.22± 0.22
5CA1341.626	6	9-9	9.00 ±0.00	0			5	0.04-1.34	0.55± 0.22
5CA1341.627	6	1-9	5.33 ±1.41	5	1-5	2.80 ±0.66	1	0.33-0.33	0.33±0.00
5CA1341.629	6	3-9	6.67 ±1.09	5	2-5	3.80 ±0.58	2	0.88-0.99	0.94± 0.06
5CA1341.630	5	7-9	8.20 ±0.49	3	0-1	0.33 ±0.33	2	0.01-0.11	0.06± 0.05
5CA1341.631	6	5-9	8.33 ±0.67	0			4	0.02-1.16	0.57± 0.26
5CA1341.632	6	3-9	7.33 ±1.09	4	1-4	2.75 ±0.75	3	0.08-1.55	0.87± 0.43
5CA1341.636	4	5-9	7.50 ±0.96	4	0-0	0.00 ±0.00	1	0.27-0.27	0.27±0.00

DH lines	Cotyledon resistance*			Adult plant resistance*			Seed weight (g/plant)		
	N**	Range	Mean ± SE	N**	Range	Mean ± SE	N**	Range	Mean ± SE
5CA1341.642	4	3-9	7.50 ±1.50	4	3-5	4.25 ±0.48	2	1.46-2.04	1.75± 0.29
5CA1341.644	6	9-9	9.00 ±0.00	2	4-5	4.50 ±0.50	4	0.29-1.39	0.74± 0.23
5CA1341.645	5	3-9	7.00 ±1.10	1	1-1	1.00	5	0.15-3.41	2.11± 0.70
5CA1341.647	6	3-9	7.33 ±0.95	0			1	0.21-0.21	0.21±0.00
5CA1341.649	6	5-9	7.33 ±0.80	0			4	0.08-2.20	0.89± 0.48
5CA1341.655	1	1-1	1.00 ±0.00	1	0-0	0.00	1	0.14-0.14	0.14±0.00
5CA1341.657	6	0-9	4.33 ±1.58	2	1-3	2.00 ±1.00	3	0.99-2.50	1.52± 0.49
5CA1341.658	8	7-9	8.25 ±0.37	3	5-5	5.00 ±0.00	2	0.06-0.32	0.19± 0.13
5CA1341.660	6	3-9	7.33 ±0.95	2	1-5	3.00 ±2.00	4	0.00-1.44	0.46± 0.33
5CA1341.662	1	3-3	3.00 ±0.00	1	0-0	0.00	1	0.11-0.11	0.11±0.00
5CA1341.663	3	3-7	5.00 ±1.15	3	0-0	0.00 ±0.00	1	0.02-0.02	0.02±0.00
5CA1341.665	6	5-9	7.33 ±0.61	6	0-0	0.00 ±0.00	2	0.01-0.07	0.04± 0.03
5CA1341.666	2	3-9	6.00 ±3.00	2	0-0	0.00 ±0.00	0		
5CA1341.672	10	9-9	9.00 ±0.00	8	0-5	1.38 ±0.63	4	0.08-0.16	0.12± 0.02
5CA1341.673	6	5-9	7.33 ±0.80	6	2-5	3.83 ±0.54	1	0.12-0.12	0.12±0.00
5CA1341.676	6	0-7	1.50 ±1.12	3	3-5	4.33 ±0.67	1	0.78-0.78	0.78±0.00
5CA1341.677	8	1-9	6.50 ±0.91	6	0-3	0.50 ±0.50	0		
5CA1341.678	6	3-7	5.33 ±0.61	4	3-5	4.25 ±0.48	3	0.06-0.82	0.34± 0.24
5CA1341.679	6	0-7	2.83 ±1.40	6	1-5	3.50 ±0.67	4	0.00-0.55	0.26± 0.13
5CA1341.680	6	3-9	6.67 ±1.20	0			6	0.54-2.19	1.37± 0.27
5CA1341.681	6	5-9	6.67 ±0.80	0			2	0.13-1.02	0.58± 0.45
5CA1341.682	6	9-9	9.00 ±0.00	0			2	0.01-1.45	0.73± 0.72
5CA1341.684	10	5-9	8.60 ±0.40	2	0-5	2.50 ±2.50	2	0.08-0.71	0.40± 0.32
5CA1341.688	12	0-9	5.00 ±1.12	11	0-2	0.45 ±0.21	1	0.20-0.20	0.20±0.00
5CA1341.689	5	9-9	9.00 ±0.00	1	0-0	0.00	5	0.00-1.96	0.75± 0.35
5CA1341.691	8	3-7	5.75 ±0.65	7	0-2	0.86 ±0.40	4	0.05-0.55	0.22± 0.11
5CA1341.692	6	0-9	3.00 ±1.61	4	4-5	4.50 ±0.29	3	0.00-0.09	0.03± 0.03
5CA1341.695	10	1-9	5.20 ±1.01	7	1-5	3.57 ±0.72	7	0.05-2.62	0.80± 0.33
5CA1341.698	6	7-9	8.33 ±0.42	2	1-1	1.00 ±0.00	0		
5CA1341.699	6	5-9	8.00 ±0.68	6	2-5	3.17 ±0.48	1	0.07-0.07	0.07±0.00
5CA1341.701	6	3-9	7.33 ±0.95	5	0-5	3.20 ±0.92	0		
5CA1341.702	10	0-9	3.40 ±0.87	9	0-5	3.11 ±0.54	6	0.03-1.15	0.43± 0.18
5CA1341.703	6	0-5	2.00 ±0.82	6	2-4	3.50 ±0.34	6	0.34-0.97	0.58± 0.10
5CA1341.704	23	0-9	3.70 ±0.55	22	0-3	0.23 ±0.15	0		
5CA1341.706	4	0-5	2.75 ±1.03	3	0-1	0.67 ±0.33	2	0.09-0.20	0.15± 0.06
5CA1341.708	8	1-9	6.50 ±1.24	3	2-5	3.67 ±0.88	5	0.17-1.22	0.57± 0.22
5CA1341.710	6	7-9	8.00 ±0.45	2	3-5	4.00 ±1.00	0		
5CA1341.711	6	9-9	9.00 ±0.00	4	1-5	3.75 ±0.95	2	0.69-1.24	0.97± 0.28
5CA1341.712	6	7-9	8.00 ±0.45	5	2-5	3.20 ±0.58	3	0.30-1.55	0.86± 0.37
5CA1341.713	7	0-9	6.86 ±1.42	1	1-1	1.00	1	1.98-1.98	1.98±0.00
5CA1341.714	6	9-9	9.00 ±0.00	2	3-5	4.00 ±1.00	6	0.10-1.64	0.80± 0.27

DH lines	Cotyledon resistance*			Adult plant resistance*			Seed weight (g/plant)		
	N**	Range	Mean ± SE	N**	Range	Mean ± SE	N**	Range	Mean ± SE
5CA1341.715	17	1-9	5.94 ±0.67	10	0-5	3.60 ±0.62	7	0.05-2.10	0.89± 0.27
5CA1341.717	6	3-7	4.67 ±0.61	6	1-5	3.67 ±0.71	5	0.10-1.34	0.72± 0.26
5CA1341.718	9	1-9	5.22 ±1.13	6	0-5	2.17 ±0.87	8	0.13-0.89	0.43± 0.11
5CA1341.720	6	3-9	5.67 ±0.84	5	2-5	3.20 ±0.49	3	0.07-0.49	0.25± 0.12
5CA1341.721	14	0-9	5.07 ±0.85	10	1-5	3.60 ±0.60	11	0.00-1.46	0.70± 0.12
5CA1341.724	17	5-9	8.65 ±0.26	8	0-5	1.75 ±0.59	6	0.08-1.00	0.31± 0.14
5CA1341.726	6	7-9	8.33 ±0.42	0			4	0.02-1.63	0.57± 0.37
5CA1341.728	10	0-9	7.00 ±1.18	5	1-5	3.60 ±0.87	4	0.43-1.53	1.04± 0.28
5CA1341.729	6	0-1	0.33 ±0.21	6	0-3	0.50 ±0.50	0		
5CA1341.733	6	7-9	8.33 ±0.42	6	3-5	4.67 ±0.33	4	0.00-0.92	0.25± 0.22
5CA1341.734	6	9-9	9.00 ±0.00	3	3-5	4.00 ±0.58	1	0.00-0.00	0.00±0.00
5CA1341.736	6	7-9	8.00 ±0.45	6	1-5	2.50 ±0.62	2	0.10-1.55	0.83± 0.73
5CA1341.750	6	0-5	2.33 ±0.80	6	0-1	0.17 ±0.17	2	0.27-0.65	0.46± 0.19
5CA1341.753	6	5-9	7.33 ±0.61	6	3-5	4.50 ±0.34	3	0.24-0.52	0.34± 0.09
5CA1341.760	6	5-9	8.00 ±0.68	3	5-5	5.00 ±0.00	0		
5CA1341.773	6	1-9	4.67 ±1.50	0			3	0.02-0.04	0.03± 0.01
5CA1341.776	6	5-9	7.00 ±0.73	2	5-5	5.00 ±0.00	0		
5CA1341.782	6	7-9	8.33 ±0.42	5	0-5	3.40 ±1.03	0		
5CA1341.786	5	9-9	9.00 ±0.00	0			2	0.00-0.03	0.02± 0.01
5CA1341.800	6	5-9	7.67 ±0.67	2	3-5	4.00 ±1.00	1	0.08-0.08	0.08±0.00
5CA1341.805	6	0-5	1.33 ±0.88	6	2-5	3.67 ±0.49	2	0.04-0.72	0.38± 0.34
5CA1341.807	5	7-9	8.60 ±0.40	1	1-1	1.00	0		
5CA1341.814	6	3-9	6.67 ±0.80	4	3-5	4.50 ±0.50	0		
5CA1341.818	5	9-9	9.00 ±0.00	1	5-5	5.00	0		
5CA1341.820	6	5-9	8.00 ±0.68	3	3-5	4.00 ±0.58	1	0.70-0.70	0.70±0.00
5CA1341.821	5	5-9	6.60 ±0.75	3	5-5	5.00 ±0.00	0		
5CA1341.826	6	0-5	1.00 ±0.82	5	2-5	3.80 ±0.58	1	0.02-0.02	0.02±0.00
5CA1341.829	6	3-9	6.33 ±0.99	3	1-5	3.00 ±1.15	0		
5CA1341.840	6	5-9	7.00 ±0.73	1	5-5	5.00	0		
5CA1341.843	6	3-9	6.67 ±0.95	1	3-3	3.00	2	0.26-1.48	0.87± 0.61
5CA1341.844	6	3-7	5.00 ±0.73	4	3-5	4.25 ±0.48	3	0.11-3.52	1.46± 1.05
5CA1341.845	6	7-9	8.67 ±0.33	1	5-5	5.00	4	0.06-0.19	0.11± 0.03
5CA1341.852	6	5-9	8.33 ±0.67	2	3-5	4.00 ±1.00	3	0.36-1.69	1.12± 0.40
5CA1341.853	6	5-9	7.33 ±0.61	3	5-5	5.00 ±0.00	0		
5CA1341.855	6	3-9	6.33 ±0.84	4	1-4	2.25 ±0.75	2	1.42-1.57	1.50± 0.08
5CA1341.857	5	0-9	6.00 ±1.67	1	0-0	0.00	1	1.45-1.45	1.45±0.00
5CA1341.863	6	0-9	3.67 ±1.50	5	2-5	4.40 ±0.60	0		
5CA1341.864	6	9-9	9.00 ±0.00	4	1-5	3.25 ±0.85	3	0.00-0.94	0.62± 0.31
5CA1341.865	5	0-9	6.80 ±1.74	0			1	0.53-0.53	0.53±0.00
5CA1341.867	6	5-9	8.00 ±0.68	0			3	0.46-1.21	0.85± 0.22
5CA1341.868	6	7-9	8.67 ±0.33	0			2	1.21-1.43	1.32± 0.11

DH lines	Cotyledon resistance*			Adult plant resistance*			Seed weight (g/plant)		
	N**	Range	Mean ± SE	N**	Range	Mean ± SE	N**	Range	Mean ± SE
5CA1341.874	6	7-9	8.67 ±0.33	0			4	0.04-0.88	0.29± 0.20
5CA1341.898	6	0-5	2.83 ±0.83	6	0-3	1.33 ±0.42	6	0.04-1.86	0.86± 0.26
5CA1341.909	5	3-9	5.80 ±1.20	3	0-4	2.00 ±1.15	5	0.00-1.41	0.82± 0.27
5CA1341.911	6	1-9	6.33 ±1.33	6	3-5	4.17 ±0.40	4	0.02-0.50	0.23± 0.11
5CA1341.914	6	0-1	0.67 ±0.21	6	0-1	0.33 ±0.21	0		
5CA1341.917	6	0-3	1.33 ±0.56	6	0-3	1.00 ±0.52	3	0.17-0.47	0.34± 0.09
Total	**822 (127)**	**0-9**	**6.40 ±0.21**	**454 (103)**	**0-5**	**2.66 ±0.17**	**351 (103)**	**0.00-2.11**	**0.61 ±0.05**

*Cotyledon resistance in 0 - 9 scale and adult plant resistance in 1 – 5 scale, where 0 = highly resistant (≈ *B. carinata*).
**'Zero' indicates that the plant(s) died.

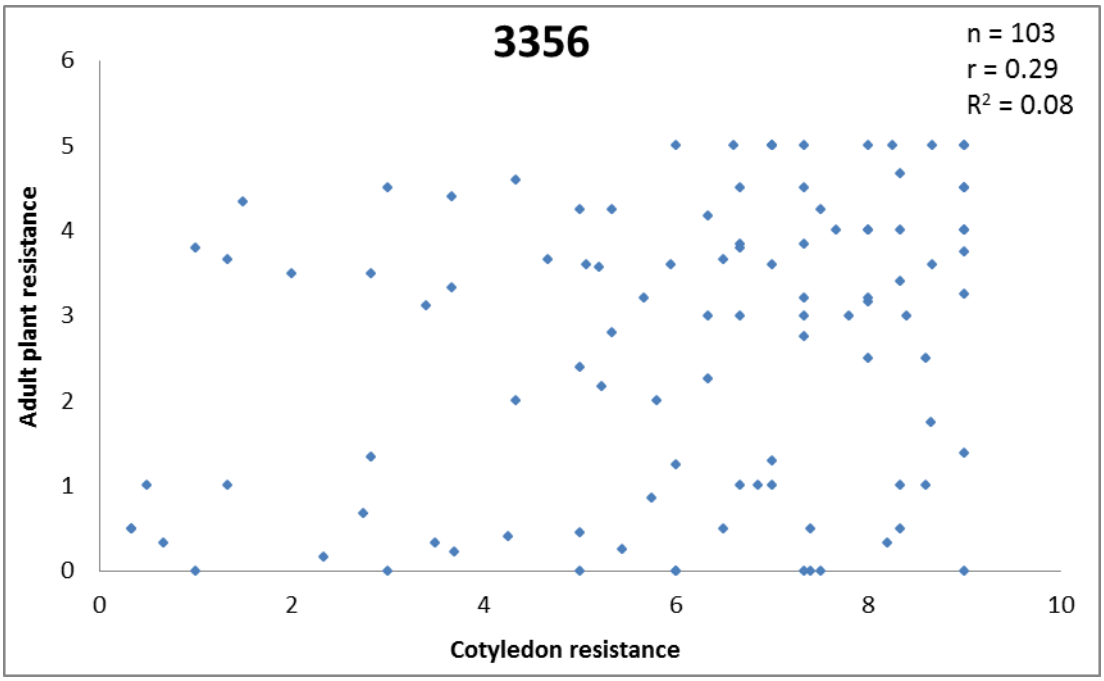

Fig. 4. Relationship between cotyledon and adult plant resistance to *Leptosphaeria maculans* PG2-type isolate #3356 in the DH population derived from advanced backcross generation plants of (*Brassica napus* cv. Polo × *B. carinata* accession #98-14513) × *B. napus* cv. Westar (PCW). *All DH lines were susceptible to PG4/PGt-type isolates#290CDN/ #BL05-08RK.*

Evaluation of the pedigree families for resistance to PG2-type isolates #3356

Thirty-seven advanced generation families (170 seedlings total) were evaluated for resistance to #3356 at adult plant stage, where only five (14%) families had disease score ≤1.0 (Table 11). The occurrence of PG2-type resistance in this pedigree population was far below when compared with the occurrence of resistance in the same population to PG4/PGt-type isolates 290CDN/BL05-08 (Table 9). Seed set in all plants of this population was poor, as was found while evaluating the same population for resistance to 290CDN/BL05-08 (Table 9).

Table 11. Adult plant responses to *L. maculans* PG2-type isolate #3356 in 37 pedigree lines derived from (*B. carinata* accession #98-14513 × *Brassica napus* cv. Westar) × *B. napus* cv. Westar (CWW) and (*Brassica napus* cv. Polo × *B. carinata* accession #98-14513) × *B. napus* cv. Westar (PCW) interspecific crosses.

Pedigree			Adult plant resistance			Seed weight (g/plant)		
Cross	Gen.	Family	N	Range	Mean ± SE	N	Range	Mean ± SE
CWW	BC_3S_2	5CA1250.322	1	0-0	0.00	0		
CWW	BC_3S_2	5CA1250.325	4	4-5	4.75 ±0.25	0		
CWW	BC_2S_3	5CA1250.362	2	5-5	5.00 ±0.00	0		
CWW	BC_2S_3	5CA1250.364	4	5-5	5.00 ±0.00	0		
CWW	BC_2S_6	5CA1250.401	3	5-5	5.00 ±0.00	1	0.22-0.22	0.22
CWW	BC_2S_6	5CA1250.598	2	2-5	3.50 ±1.50	0		
PCW	BC_3S_1	5CA1341.552	3	0-0	0.00 ±0.00	2	0.01-0.01	0.01 ±0.00
PCW	BC_3S_1	5CA1341.553	6	4-5	4.83 ±0.17	2	0.01-0.01	0.01 ±0.00
PCW	BC_3S_1	5CA1341.554	4	0-5	2.00 ±1.08	0		
PCW	BC_3S_1	5CA1341.555	8	0-5	3.00 ±0.76	2	0.06-0.09	0.08 ±0.02
PCW	BC_3S_1	5CA1341.556	4	0-2	1.00 ±0.41	1	0.04-0.04	0.04
PCW	BC_3S_1	5CA1341.557	4	0-3	1.75 ±0.63	0		
PCW	BC_3S_1	5CA1341.559	6	1-5	2.83 ±0.60	3	0.01-0.04	0.02 ±0.01
PCW	BC_3S_1	5CA1341.560	7	0-5	2.14 ±0.83	3	0.01-0.13	0.06 ±0.04
PCW	BC_3S_1	5CA1341.561	5	1-5	2.80 ±0.92	1	0.04-0.04	0.04
PCW	BC_3S_1	5CA1341.562	8	0-5	2.25 ±0.75	0		
PCW	BC_3S_1	5CA1341.563	6	0-5	1.67 ±0.84	0		
PCW	BC_3S_1	5CA1341.564	7	0-5	1.43 ±0.69	2	0.01-0.07	0.04 ±0.03
PCW	BC_3S_1	5CA1341.565	4	0-5	2.50 ±1.44	0		
PCW	BC_3S_1	5CA1341.566	8	1-5	3.88 ±0.58	3	0.01-0.13	0.07 ±0.03
PCW	BC_3S_1	5CA1341.567	8	1-5	2.88 ±0.67	1	0.11-0.11	0.11
PCW	BC_3S_1	5CA1341.568	7	0-5	1.43 ±0.69	2	0.01-0.01	0.01 ±0.00
PCW	BC_3S_1	5CA1341.569	3	0-2	0.67 ±0.67	0		
PCW	BC_3S_2	5CA1341.925	4	0-3	1.25 ±0.63	0		
PCW	BC_3S_2	5CA1341.943	4	0-5	2.75 ±1.31	1	0.01-0.01	0.01
PCW	BC_3S_2	5CA1341.945	3	2-3	2.33 ±0.33	1	0.01-0.01	0.01
PCW	BC_3S_2	5CA1341.953	3	3-4	3.67 ±0.33	2	0.01-0.06	0.04 ±0.03
PCW	BC_3S_2	5CA1341.956	4	3-5	4.50 ±0.50	0		
PCW	BC_3S_2	5CA1341.964	4	0-5	3.75 ±1.25	2	0.02-0.14	0.08 ±0.06
PCW	BC_3S_2	5CA1341.967	4	4-5	4.50 ±0.29	0		

Pedigree			Adult plant resistance			Seed weight (g/plant)		
Cross	Gen.	Family	N	Range	Mean ± SE	N	Range	Mean ± SE
PCW	BC_3S_2	5CA1341.969	4	5-5	5.00 ±0.00	0		
PCW	BC_3S_2	5CA1341.970	4	1-5	3.25 ±0.85	0		
PCW	BC_3S_2	5CA1341.971	2	2-5	3.50 ±1.50	0		
PCW	BC_3S_2	5CA1341.972	2	0-0	0.00 ±0.00	0		
PCW	BC_3S_2	5CA1341.973	7	0-4	1.57 ±0.61	1	0.07-0.07	0.07
PCW	BC_3S_2	5CA1341.974	7	0-5	2.43 ±0.65	3	0.01-0.12	0.07 ±0.03
PCW	BC_3S_2	5CA1341.976	4	0-1	0.50 ±0.29	0		
Total			**170 (37)**		**1.53 ±0.25**	**33 (18)**		**0.05 ±0.01**

Summary and conclusions

A total of 661 DH lines were produced from advanced backcross generation plants of (*Brassica napus* × *B. carinata*) × *B. napus* interspecific cross of which 405 lines were tested for cotyledon (5,466 seedlings in total) and adult-plant (4,792 plants in total) resistance to PG4/PGt-type isolates 290CDN/BL05-08. Adult plant resistance was found in more than 20% of the DH lines, while the occurrence of cotyledon resistance in this population was low (about 2%). The occurrence of adult-plant resistance in 77 pedigree families (682 plants in total) was also similar to the level of resistance found in the DH population. The chromosome B3 or B7 alone was not capable of conferring adult-plant resistance to the PG4/PGt-type isolates 290CDN/BL05-08; however, presence of these two chromosomes exerted greater resistance in the plants.

To diversify the PG2-type resistance in canola, the DH lines and pedigree families which are susceptible to PG4/PGt-type isolates 290CDN/BL05-08 were tested (822 at cotyledon stage and 524 at adult-plant stage) for resistance to a PG2-type isolate #3356 for the development of resistant lines carrying genome content introgressed from the B genome of *B. carinata*. The occurrence of adult-plant resistance in these populations was about 15-20%; however, seed set in this population was very poor – apparently due to the presence of B chromosomes. Correlation between cotyledon and adult-plant for resistance to #3356 was r = 0.29, which is much weaker than the correlation found between these two traits for resistance to the PG4/PGt-type isolates 290CDN/BL05-08 (r = >0.60). This also indicates that the genetic control of cotyledon and adult-plants resistance to these isolates is very different.

A total of 347 resistant plants belonging to 129 DH lines or pedigree families are selected based on blackleg resistance and seed set under self-pollination for field and greenhouse tests in 2016 for evaluation of fertility of the plants/lines, and their agronomic and seed quality traits as well as resistance to blackleg disease under field condition (PG3/PG4-type isolates prevalent) in Manitoba.

Implications for Alberta's agriculture

Canola (*B. napus*) is more than CAN$ 3 billion industry in Canada where Alberta's share is about one-third. This crop contributes to about CAN$ 19 billion economic activity in this country when all indirect benefits are included. Crop losses due to blackleg alone can be up to 15-20%. Durable resistance to blackleg disease in canola cultivars will therefore secure more than CAN$ 0.5 billion for the Canadian canola industry (more than CAN$ 0.17 billion in Alberta).

With the global climate change, it is anticipated that our crop plants will suffer from different abiotic and biotic stresses including infestation caused by diseases and insect pests to a greater extent than the threats we face today. Therefore, the development of disease resistant cultivars will secure sustainable production of canola on the Canadian prairies. Moreover, breeding for disease resistance is also considered to be the more environmental friendly way of managing this crop. The blackleg resistant germplasm developed and the knowledge gained from this project can be used for the development of euploid ($2n$ = 38 chromosomes) *B. napus* canola lines carrying a new source of resistance, and this will diversify the crop for the resistance genes. Existence of genetic diversity in crop cultivars, especially for resistance genes, is critical for secured production of the crop while pathogen population is changing.

Targets achieved compared to those contemplated

The two main objectives of this project was to identify the B-genome chromosome(s) carrying resistance to blackleg PG4/PGt-type isolate 290CDN/BL05-08 through molecular cytogenetic study and application of doubled haploid (DH) technique, and to introgress resistance from the B-genome chromosome(s) of *B. carinata* into the A/C-genome of *B. napus*. Introgression of

resistance into *B. napus* through recurrent backcrossing was found to be uncertain due to the involvement of more than one chromosome (at least B3 and B7) in the control of this trait and for the challenges associated with introgression of a gene from the B-genome chromosome into the A/C genome chromosome. Therefore, we focused on self-pollination of the populations developed in this project as a strategy for introgression of resistance into *B. napus*. Through this approach, we identified 343 (43 + 280 + 20) lines/plants resistant to #290CDN/BL05-08 and four plants/lines resistant to #3356 at adult plant stage (score 0-1) with seed set of >0.2 g/plant. Molecular marker analysis indicates that some of the plants/lines may lack the B genome chromosomes; however, low seed set indicate that, at least, fragments of B chromosome(s) might be present in these plants. Thus, given the complexity of this research project, we made significant progress towards introgression of PG4/PGt-type resistance from the B genome of *B. carinata* into *B. napus* canola. Continued effort on the resistant lines/plants expected to result euploid (2*n* = 38 chromosomes) *B. napus* lines carrying resistance to these isolates.

Future works

The following activities currently undertaken for continuation with the research results obtained from this project:

(i) A total of 43 lines, derived through self-pollination of single plants of 21 DH lines, lacking detectable B genome chromosome(s) by use of molecular markers but showed resistance to blackleg isolates 290CDN/BL05-08 (adult plant score 0-1), were selected for evaluation of fertility (seed set under self-pollination; good seed will indicate the lack of B genome chromosome in these plants) in greenhouse in summer 2016. Seed increase of these lines will also be done for further testing.

(ii) A total of 284 lines, derived through self-pollination of single plants of 88 DH lines with seed set 0.21 to 2.89 g/plant (mean = 0.53 g/plant), will be evaluated in field nursery plots in 2016 for agronomic and seed quality traits. Of the 284 lines, 195 had disease score of ≤0.56 for adult-plant resistance. Evaluation for plants fertility and seed increase of these 284 lines will be done in greenhouse in summer 2016.

(iii) Forty-one of the above-mentioned lines, with disease score of 0 to 1 (mean = 0.05) for adult-plant resistance to 290CDN/BL05-08 and high seed set (mean = 1.65 g/plant under self-pollination), will be evaluated in a field in Manitoba where cultivars carrying PG2-type resistance found to be completely susceptible. This work will be carried out in collaboration with the University of Manitoba (Dr. Dilantha Fernando).

(iv) A total of 20 BC_3F_3/BC_2F_6 plants resistant to 290CDN/BL05-08 were selected for further study. Seed set on these plants was low (0.06-0.42 g/plant; mean = 0.13 g/plant); therefore, it is highly likely that they carry the B-genome chromosome(s) associated with resistance. Progeny of these plants will be grown in greenhouse in 2016 summer for introgression of resistance into *B. napus* chromosomes through self-pollination.

(v) Based on results from the above-mentioned field and greenhouse (a total of 754 plants will be grown in greenhouse in summer 2016) tests, future strategy will be laid out.

(vi) It is also planned to test a set of the above-mentioned resistant lines in Australia where highly virulent blackleg pathotypes are prevalent.

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Appendix I

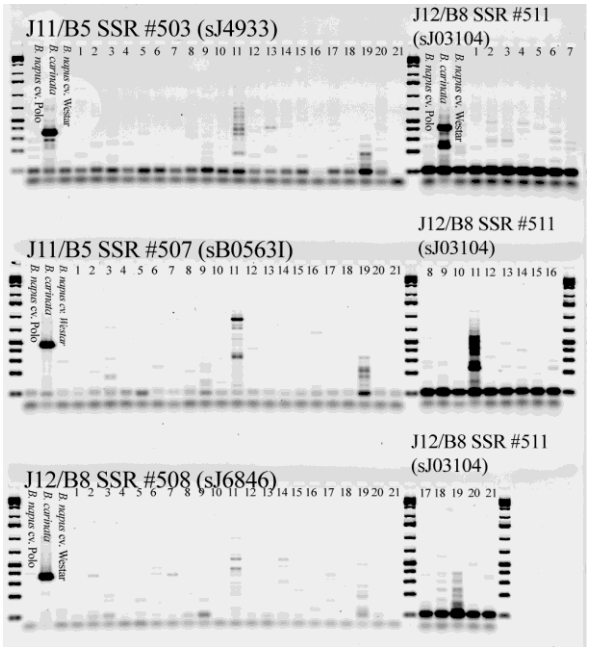

Fig S1. Agarose gel electrophoresis of PCR amplified products of 21 DH plants with B5 (J11) SSR markers # 503 and 507 and B8 (J12) SSR markers #508

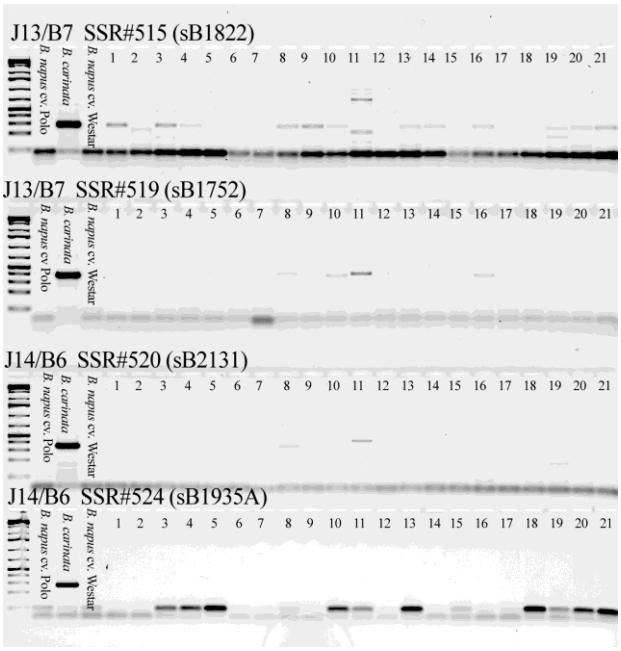

Fig S2. Agarose gel electrophoresis of PCR amplified products of 21 DH plants with B7 (J13) SSR markers # 515 and 519 and B6 (J14) SSR markers # 520 and 524

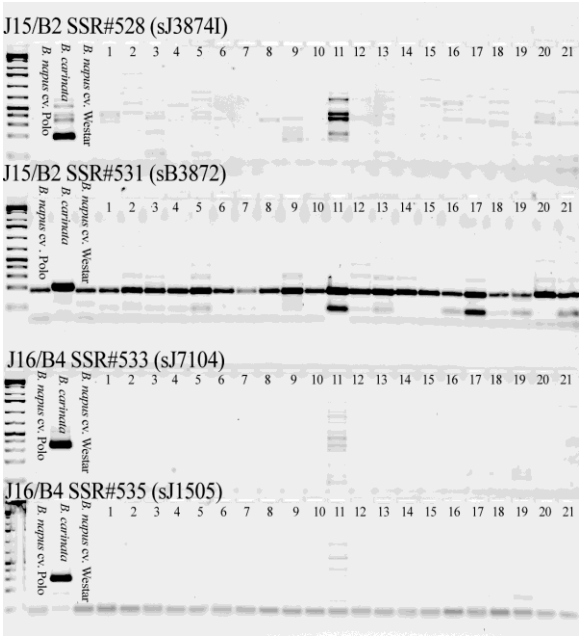

Fig S3. Agarose gel electrophoresis of PCR amplified products of 21 DH plants with B2 (J15) SSR markers# 528 and 531 and B4 (J16) SSR markers # 533 and 535

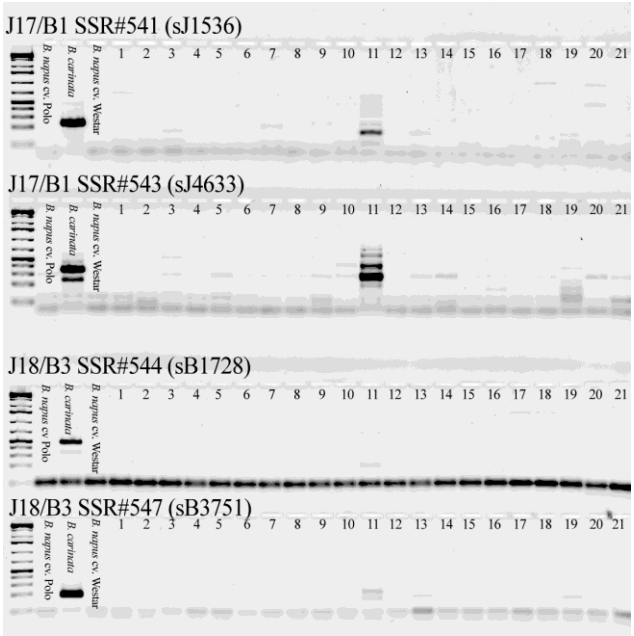

Fig S4. Agarose gel electrophoresis of PCR amplified products of 21 DH plants with B1 (J17) SSR markers # 541 and 543 and B3 (J18) SSR markers #544 and 547

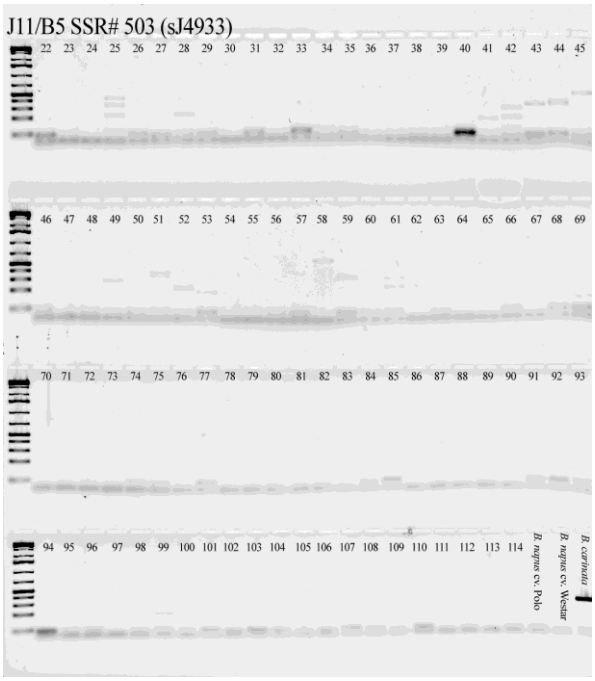

Fig S5. Agarose gel electrophoresis of PCR amplified products of 93 DH plants with B5 (J11) SSR# 503

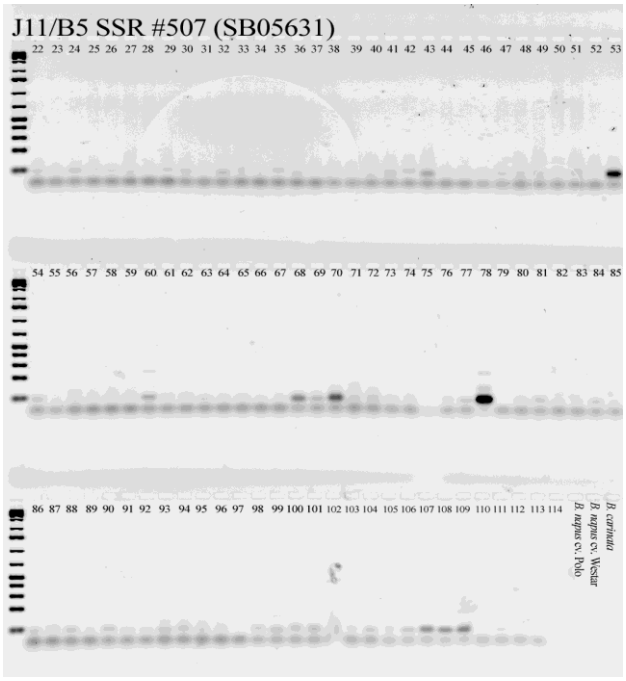

Fig S6. Agarose gel electrophoresis of PCR amplified products of 93 DH plants with B5 (J11) SSR# 507

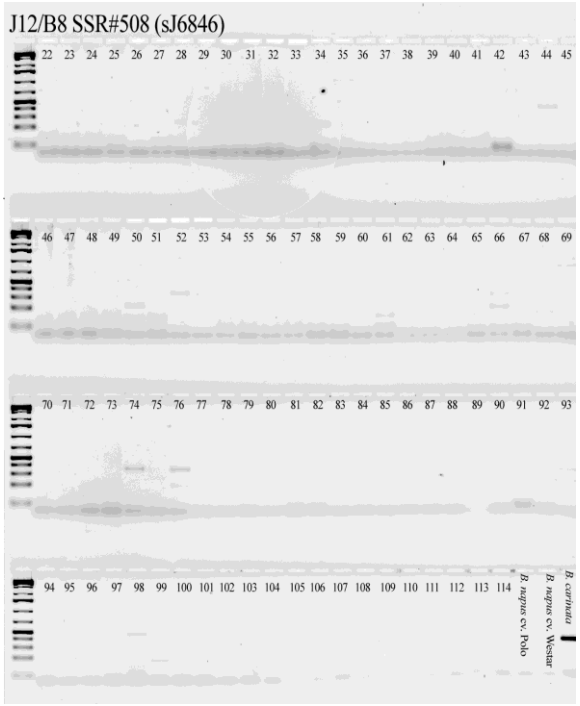

Fig S7. Agarose gel electrophoresis of PCR amplified products of 93 DH plants with B8 (J12) SSR# 508

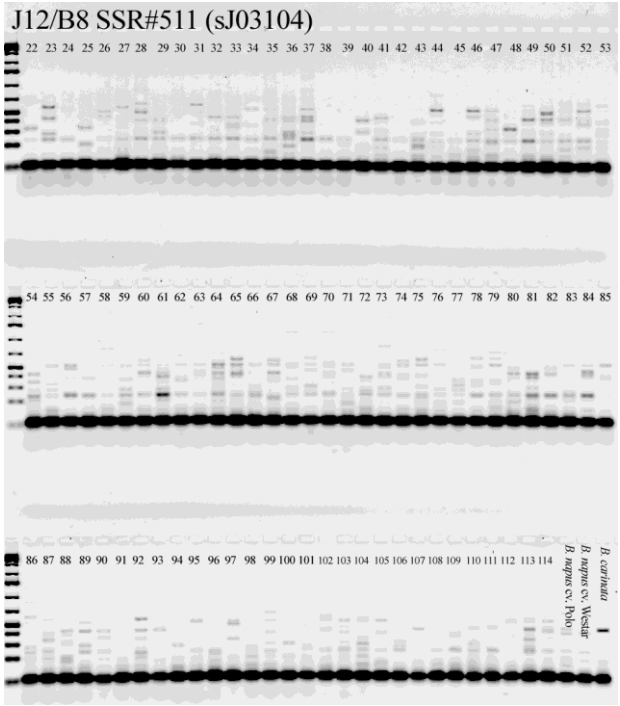

Fig S8. Agarose gel electrophoresis of PCR amplified products of 93 DH plants with B8 (J12) SSR# 511

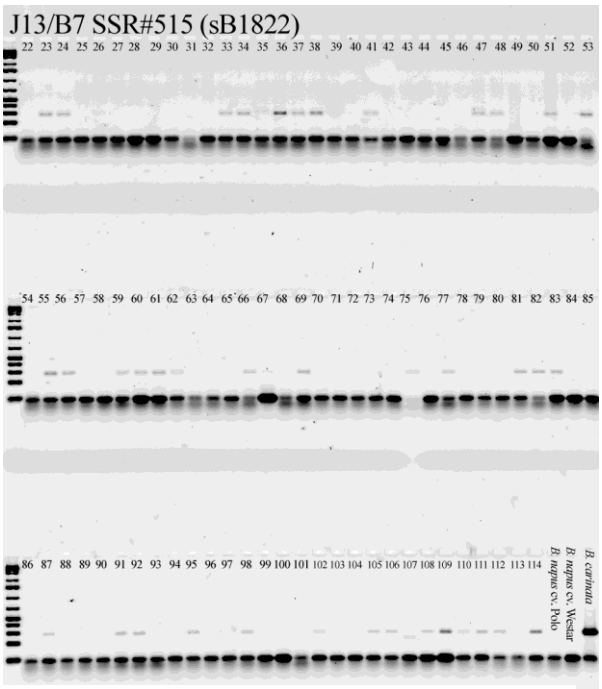

Fig S9. Agarose gel electrophoresis of PCR amplified products of 93 DH plants with B7 (J13) SSR# 515

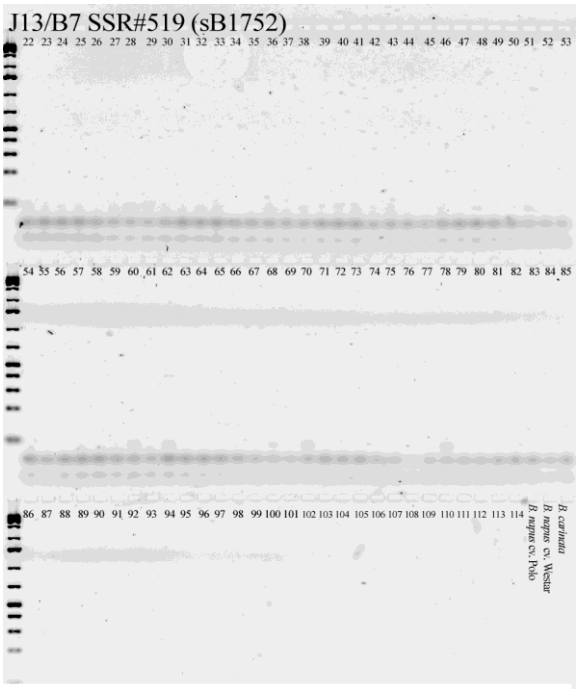

Fig S10. Agarose gel electrophoresis of PCR amplified products of 93 DH plants with B7 (J13) SSR# 519

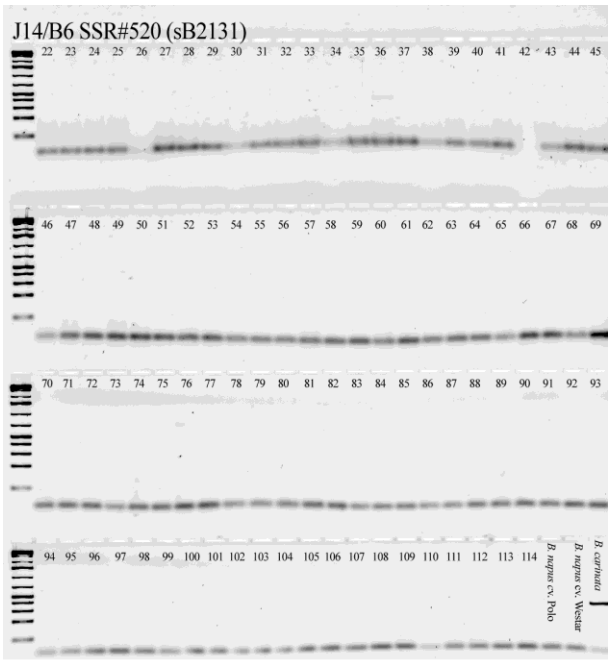

Fig S11. Agarose gel electrophoresis of PCR amplified products of 93 DH plants with B6 (J14) SSR# 520

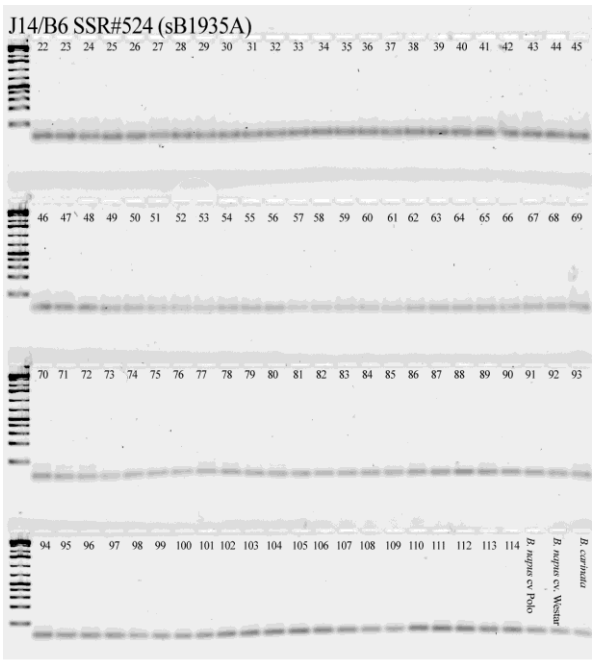

Fig S12. Agarose gel electrophoresis of PCR amplified products of 93 DH plants with B6 (J14) SSR# 524

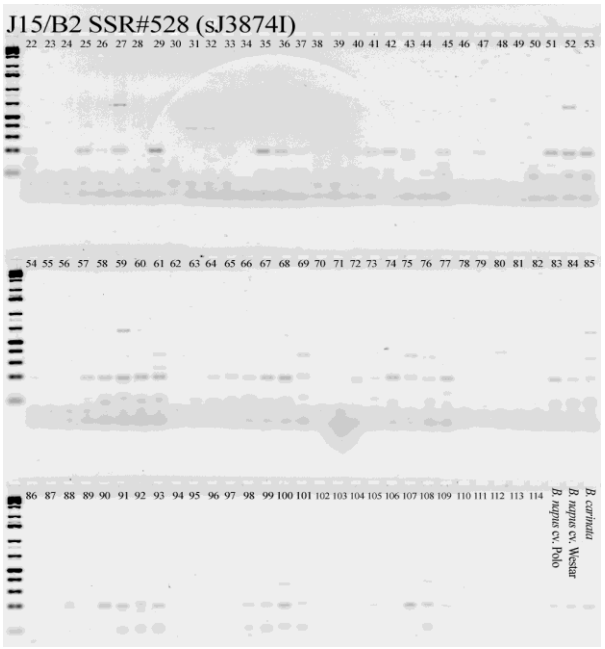

Fig S13. Agarose gel electrophoresis of PCR amplified products of 93 DH plants with B2 (J15) SSR# 528

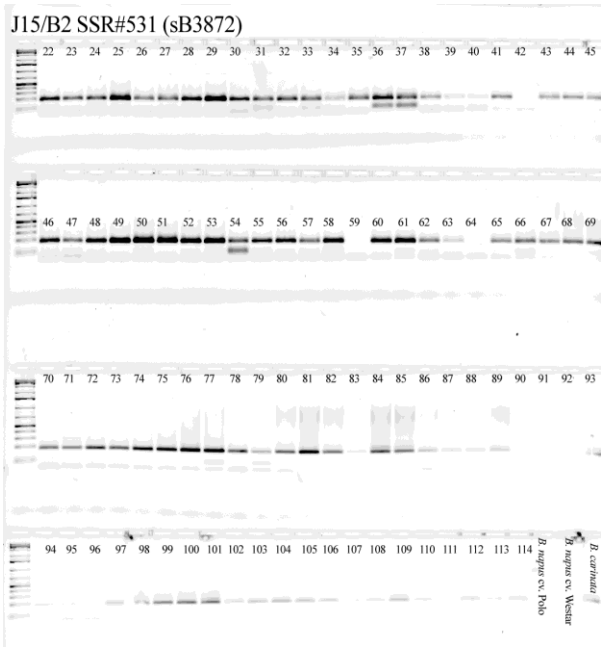

Fig S14. Agarose gel electrophoresis of PCR amplified products of 93 DH plants with B2 (J15) SSR# 531

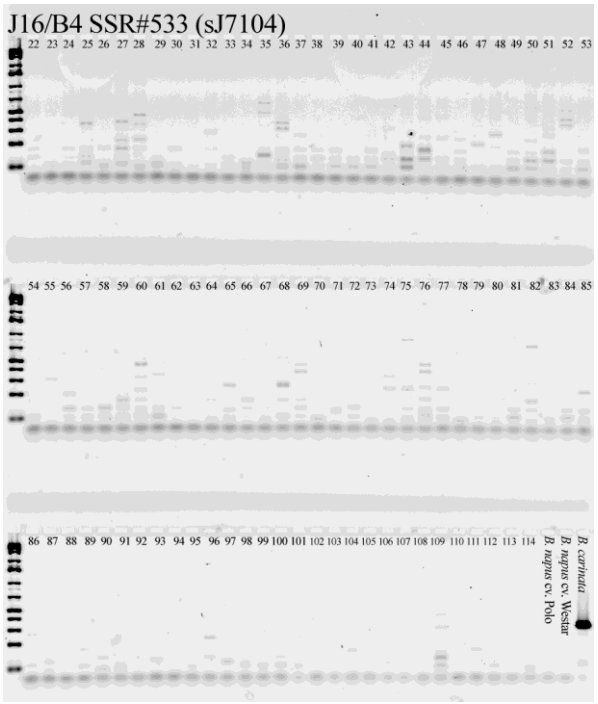

Fig S15. Agarose gel electrophoresis of PCR amplified products of 93 DH plants with B4 (J16) SSR# 533

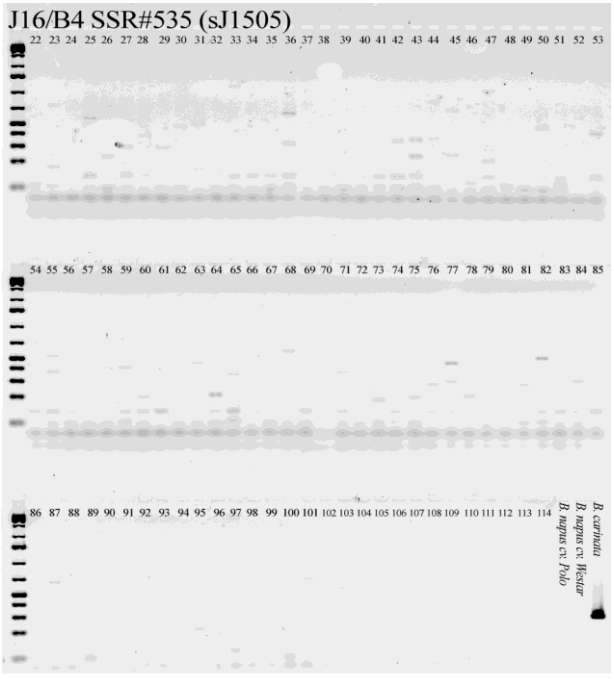

Fig S16. Agarose gel electrophoresis of PCR amplified products of 93 DH plants with B4 (J16) SSR# 535

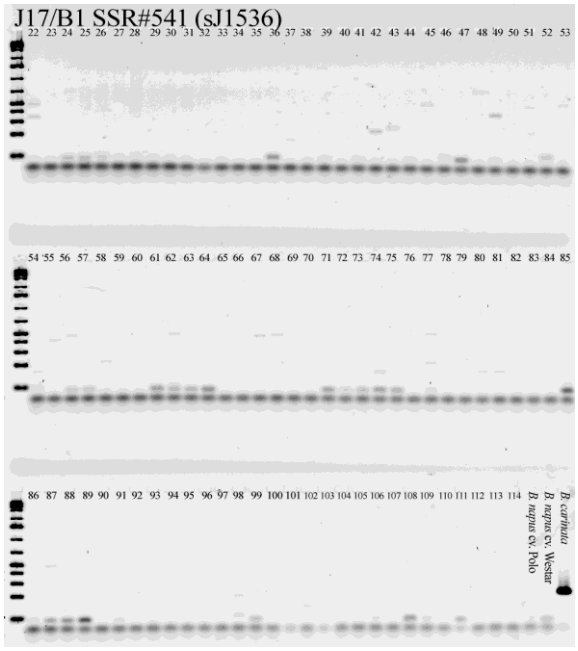

Fig S17. Agarose gel electrophoresis of PCR amplified products of 93 DH plants with B1 (J17) SSR# 541

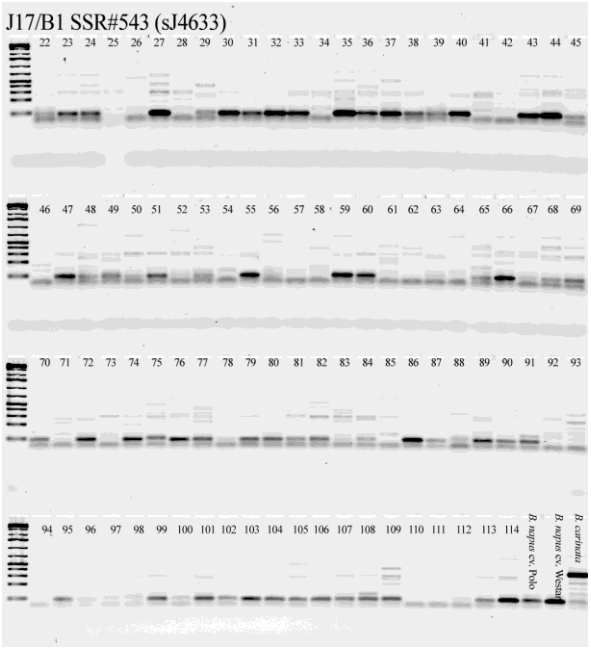

Fig S18. Agarose gel electrophoresis of PCR amplified products of 93 DH plants with B1 (J17) SSR# 543

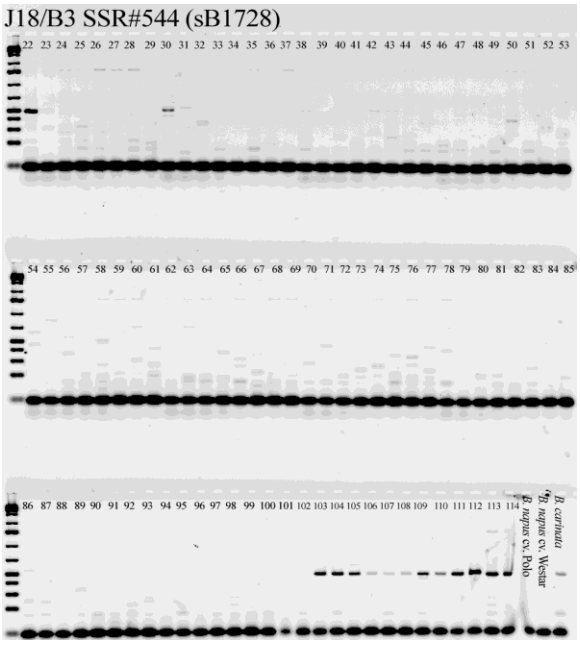

Fig S19. Agarose gel electrophoresis of PCR amplified products of 93 DH plants with B3 (J18) SSR# 544

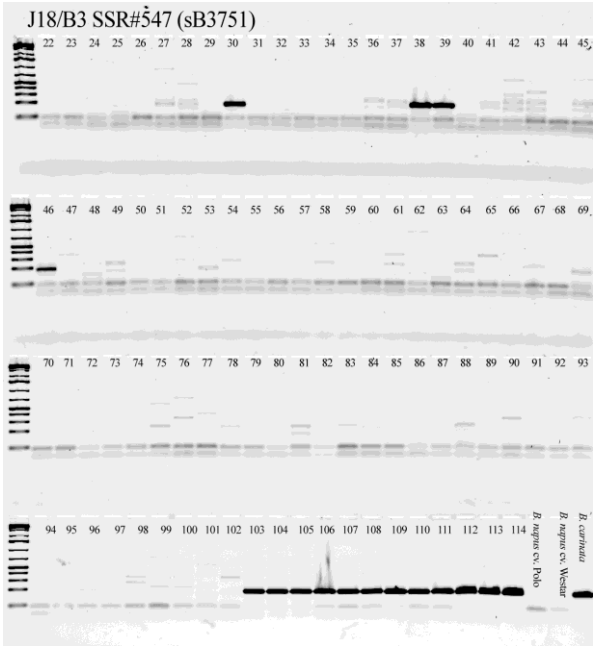

Fig S20. Agarose gel electrophoresis of PCR amplified products of 93 DH plants with B3 (J18) SSR# 547