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Project: Identification of herbicide-tolerant canola breeding lines with resistance to blackleg

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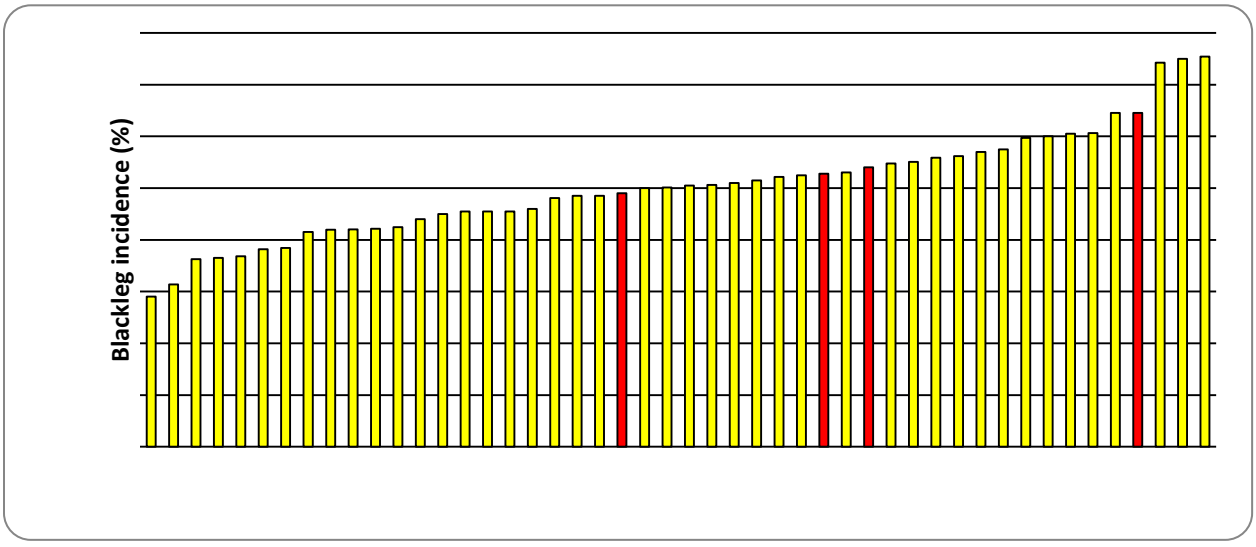
Forty five *B. napus* breeding lines with tolerance to the herbicide glyphosate were evaluated in a field trial in Langdon in the summer of 2012. These are advanced breeding lines from the canola breeding program at NDSU. The nursery was supplemented with blackleg-infected canola stems collected from commercial fields in 2011. The lines were planted following a randomized complete block design with four replications, but due to a misdirected application of a herbicide, the nursery had to be replanted in early June 2012. In spite of the late replanting, or perhaps because of it, moderate disease pressure was observed in the study (Figure 1). Disease incidences in the 2012 nursery ranged between 29 and 75% and severity between 0.4 and 3.9. Under these conditions, none of the materials evaluated were immune to blackleg. Line 9067 (BL-17) had the lowest blackleg incidence with just under 30% incidence, value that was significantly lower (P=0.05) than that of three of the commercial controls included in the trial. This same line outperformed commercial checks in blackleg nurseries in the past three years.

Figure 1. Blackleg incidence on elite *B. napus* breeding lines in Langdon in 2012. Commercial controls are in red bars. Least significant difference = 23% (P=0.05).



Another line, 12-AYT-1Hy-3 also had significantly less disease and higher yields than three of the four commercial controls. This is the first time this entry is being evaluated. The

identification of lines that outperformed three of the four commercial controls is an indication of the progress achieved by the breeding program.

The results of the field screening highlight the need for more effective sources of resistance. In this project, our goal was to identify sources of resistance against blackleg as well as markers associated with it in the USDA collection of *B. juncea* plant introductions. To that effect, we have finished the screening of 298 *B. juncea* accessions for their reaction to pathogenicity groups (PG) 2, 3, 4, and T. These accessions were evaluated in three different instances in greenhouse conditions. In each instance, every accession was replicated three times and in each replication three plants were evaluated. All accessions were inoculated at the seedling stage using a mixture of spores produced by five isolates belonging to each PG. Screening of each group was conducted separately from the others. Table 2 presents the most promising accessions.

Table 2. *Brassica juncea* plant introduction materials identified as resistant to pathogenicity groups (PG) 2, 3, 4, and T of *Leptosphaeria maculans* in greenhouse conditions.

	Median disease severity			
Accession	PG-2[1]	PG-3	PG-T	PG-4
PI 175100	4	6.0	-[2]	-
PI 426375	2	4.5	-	-
PI 426384	5	3.5	-	-
PI 426385	4	3.5	-	-
PI 459007	3	2.5	-	-
Ames 9914	-	-	3.0	2.0
PI 179858	-	-	2.2	2.8
PI 311726	-	-	1.8	3.3
PI 426316	-	-	2.5	3.0
PI 426320	-	-	2.0	2.0
PI 426330	-	-	2.5	2.5
PI 649109	-	-	2.5	3.0
PI 649113	-	-	1.5	3.0
PI 649123	-	-	2.0	2.0

[1] PG= pathogenicity groups
[2] Accessions were screened but their reaction values are not presented

Accessions considered most resistant to PG-2 were also resistant to PG-3 whereas accessions considered most resistant to PG-4 were also resistant to PG-T. Most accessions resistant to the latter two groups were very susceptible to PG-2 and PG-3; however, some materials that were considered resistant to PG-2 and PG-3 were moderately resistant to PG-T and PG-4. Association mapping analysis was used to identify 13 DArT markers that were significantly associated with resistance to PG-2 (Table 3); three of them also were associated with resistance to PG-3. The combined presence of five of the markers associated with PG-2 helped explain approximately 15% of the phenotypic variability observed whereas the markers associated with resistance to PG-3 explained approximately 4% of the phenotypic variability. None of the markers used in this study, however, could be associated with resistance to PG-4 or PG-T. Since the markers used in

this study were located in the A genome we believe markers associated with resistance to PG-T and PG-4 may be located in the B genome.

Table 3. DArT markers associated with resistance to PG-2 isolates of *L. maculans* in *B. juncea*

Markers	*P*- value	*pFDR*	R^2
brPb_807842	<0.0001	0.019	0.94
brPb_807978	0.0001	0.019	2.74
brPb_808436	0.0001	0.019	2.74
brPb_809966	0.0001	0.019	2.74
brPb_809516	0.0005	0.041	2.34
brPb_807813	0.0006	0.041	2.11
brPb_808071	0.0006	0.041	1.14
brPb_660465	0.0007	0.041	5.94
brPb_659520	0.0007	0.041	3.26
brPb_660505	0.0007	0.041	3.26
brPb_660716	0.0007	0.041	3.26
brPb_809224	0.0007	0.041	3.26
brPb_657781	0.0007	0.041	3.26

Breeding populations will be developed using the accessions identified in this study. Crosses will be made in such way that resistance to all pathogenicity groups could be pyramid into single lines. At the same time efforts will be made to transfer this resistance into commercial *B. napus* genotypes. These activities are the topic of a new three year project proposal that was recently submitted for funding.

The genetics of 605 *Leptosphaeria maculans* isolates collected from several canola producing regions of North Dakota were characterized using seven microsatellite and four minisatellite markers as well as markers associated with two mating types. Presence of both mating types is required for sexual recombination (ascospore production) to occur in the population. The populations were divided into five geographic regions. Results of the study showed a significant departure from the expected 1:1 ratio of mating types in three of the five regions studied indicating a possibly dominant role of pycnidiospores in development of blackleg epidemics in the state. This hypothesis was supported by recent migration analysis and the indices of population differentiation (G''_{ST}) and genetic identity (*I*).