Breeding for Blackleg Disease Resistance in Canola:
The Impact of New Strains on the Industry

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Rapeseed/canola is the third most important source of vegetable oil in the world, after soybean and palm oil (Beckman, 2005). During the past twenty years, it has surpassed peanut, cottonseed, and most recently, sunflower, in worldwide production. Canola/rapeseed is the major oilseed crop grown in western Canada, grown annually on 4 to 5 million ha. Canola/rapeseed production is valued at approximately $2.5 billion annually, second in annual value only to wheat.

Blackleg, caused by the fungus *Leptosphaeria maculans* (Desm.) Ces. & de Not. (anamorph *Phoma lingam* Tode ex Fr.), is a devastating disease present all over rapeseed/canola growing areas except China (West et al., 2001). The pathogen can infect all parts of the plant, but stem canker is the most serious symptom as it causes plant lodging and yield loss. The disease control methods such as crop rotation (Guo et al., 2005), resistant cultivars (Fernando et al., 2001) and seed treatment with fungicide (Fernando, and Nakkeeran, unpublished data), have proven to be effective.

Leptosphaeria maculans isolates can be categorized into several pathogenicity groups (PG) based on interaction phenotype (IP) of the isolates with different avirulence (*Avr*) genes on the cotyledons of three *Brassica napus* differential cultivars Westar (susceptible), Glacier (*Rlm2* and *Rlm3* resistant genes) and Quinta (*Rlm1*and *Rlm4* linked resistant genes) (Mengistu et al., 1991). PG2 (virulent on Westar) is prevalent in Western Canada, PG3 (virulent on Westar and Glacier) and PG4 (virulent on Westar, Glacier and Quinta) are mainly present in Western Europe and Australia. Recently PGT (virulent on Westar and Quinta) was added into PGs (Chen and Fernando, 2005). The spore movement from one field to another may help spread the new strains (Guo and Fernando, 2005).

PG identification is based on gene-for-gene relationship, where the IP (resistance or susceptibility) depends on the presence of one major gene for resistance (*Rlm*) in the plant and one corresponding avirulence (*Avr*) gene in the pathogen. For instance, dominant single resistant allele *Rlm2* in Glacier is in response to *Avr* gene *Avrlm2* in PG2 isolates of *L. maculans*, resulting in an incompatible interaction between var. Glacier and PG2 isolates. Pair matching between linked *Rlm1* and *Rlm4* genes in Quinta and

unlinked *Avrlm1* and *Avrlm4* in *L. maculans* isolates controls the "Quinta-PG2" interaction. A gene match between *Rlm1* in Quinta and its complementary *Avrlm1* in PG3 isolates determines the "Quinta-PG3" incompatible interaction (Ansan-Melayah et al., 1998).

PG3 and PG4 isolates were first identified during 2002 to 2004 in Western Canada and North Dakota (Bradley et al., 2005; Chen and Fernando, 2005; Fernando and Chen, 2003). Since then the focus has been on the potential risk of these new isolates to the canola industry in Canada. Although the varieties that are released in Western Canada have MR or R-rating for Blackleg, almost all have been bred for resistance against the predominant PG2 strains. Therefore resistance background (genes governing resistance) of Canadian cultivars is not well characterized or known.

Genetic resistance to *L. maculans* includes two types: monogenetic (qualitative, single major gene), normally expressed at seedling stage and quantitative (polygenic), partial resistance expressed at the adult stage. Genetic resistance is often derived from intraspecific and interspecific breeding, especially by introgression of resistant genes from other *Brassica* species with the B genome like mustard *B. nigra*(2n=16, BB), *B. juncea* (2n=36, AABB) and *B. carinata* (2n=34, BBCC). Until now, a total of 14 single resistant loci, named *LEM1, LmF1, LmR1, LepR1, LepR2,* and *Rlm1 to Rlm9,* have been identified in the *Brassica* genome. Some of these such as *LEM1* and *LmR1* have been mapped in *B. napus* but none has been cloned yet because of the highly duplicated regions in *Brassica* genomes. A single locus *LEM1* in *B. napus* cv. Major is mapped to linkage group 6 using RFLP markers on F1-derived DH populations (Ferreira et al., 1995). *LmF1* is a single major gene controlling adult resistance in French cv. Cresor of *B. napus* (Dion et al., 1995). *LmR1* is detected in the Australian *B. napus* cvs. Shiralee and Maluka (Mayerhofer et al., 1997). *LepR1*, a single dominant allele introgressed from *B. rapa* subsp. *sylvestris,* was found to have resistance to PG2, PG3 and PG4, and *LepR2*, an incomplete dominant resistance derived from *B. rapa* subsp. *sylvestris*, is characterized with resistance to PG2 and PG3 (Yu et al., 2005). *Rlm1* is in *B. napus* cv. Quinta having resistance to PG3 (Balesdent et al., 2001). *Rlm2* is in *B. napus* cv. Glacier controlling resistance to PG2 (Ansan-Melayah et al., 1998).*Rlm3***,** a single dominant allele derived from Glacier, confers resistance to European races (Balesdent et al., 2002). *Rlm4***,** controlling the resistance in European *B. napus* cv. Jet Neuf, is found to be linked with *Rlm1* in Quinta (Balesdent et al., 2001). *Rlm5* is derived from Indian mustard (*B. juncea*) line 150-2-1, 151-2-1 and cv. Picra, conferring resistance to Australian isolates (Balesdent et al., 2002). *Rlm6*,like Rlm5, is also derived from mustard cv. Picra

(Balesdent et al., 2002). *Rlm7* is identified from *B. napus* and linked to *Rlm3*, conferring resistance to PG4 (Balesdent et al., 2002). *Rlm8* is present in *B. rapa* and conditions the resistance to PG4 (Balesdent et al., 2002). *Rlm9* is a resistance gene in *B. napus* cv. Darmor against PG4 (Delourme et al., 2004). Five dominant single resistance genes *Rlm1, Rlm3, Rlm4, Rlm7* and *Rlm9* were shown to be clustered on linkage group 10 and other genes mapped to linkage group 16 (Howlett, 2004).

On the pathogen side, ten *Avr* genes, *alm1*, *Avrlm1* to *Avrlm9* have been genetically characterized, but none has been cloned. Only *alm1* has been mapped at the University of Wisconsin (Pongam et al., 1998) and INRA, by T. Rouxel's group, attempting to isolate *Avrlm1* by map-based cloning. Of ten *Avr* genes, six genes, *Avrlm2, Avrlm3, Avrlm5, Avrlm6, Avrlm7* and *Avrlm8*, are found genetically unlinked on *L. maculans* genome. *Avrlm2* and *Avrlm3* are genetically independent but both cause incompatibility on cv. Glacier. Independent loci *Avrlm5* and *Avrlm6* induce incompatibility on *B. juncea* as it has the corresponding genes *Rlm5* and *Rlm6*. *Avrlm7* and *Avrlm8*, independent loci present in PG4 isolate, recognize the resistant source in *B. napus* and *B. rapa*, respectively.

Avr gene clusters like "*Avrlm1-Avrlm2-Avrlm6*" (they are not alleles but tightly linked and localized at *Avrlm1* region, *Avrlm6* is between *Avrlm1* and *Avrlm2*) and "*Avrlm3-Avrlm4-Avrlm7*" (it is not sure yet if they are different allelic forms of one single locus) are genetically linked at specific loci (Balesdent et al., 2002). Eighteen linkage groups in the *L. maculans* maps have been identified and at least four independent regions of the *L. maculans* genome, i.e. *AvrLm1-AvrLm2-AvrLm6* cluster, the *AvrLm3-AvrLm4-AvrLm7* region, *AvrLm5*, and *AvrLm8* are found to be responsible in host specificity (Balesdent et al., 2002). It should be made aware that one single isolate may possess more than one *Avr* gene. In other words, the isolates belonging to the same PG could be found to be polymorphic at *Avr*loci. For instance, a PG2 isolate PHW1245 possess at least *Avrlm1, Avrlm2, Avrlm4*, and *Avrlm5─Avrlm8*; isolate IBCN18 contains four *Avr* alleles *Avrlm1, Avrlm2, Avrlm4* and *Avrlm7*; and European PG4 isolates normally possess four *Avr* alleles *Avrlm5-Avrlm8* (Balesdent et al., 2002), which causes complexities in genetic analysis of *Avr* genes. The cluster "*Avrlm1-Avrlm2-Avrlm6"* is intensively being studied in France for three reasons: Firstly this cluster has been molecularly identified and localized on the genetic map. Secondly *Rlm6* derived from *B. juncea* has never been used in Europe. Finally *L. maculans* isolates have the size polymorphism of the chromosome harboring this gene cluster.

Reasonable deployment of resistance genes may avoid strong selection pressure exerted on the pathogen population to ensure the stability and effective application of the resistance. There is evidence both from Europe and Australia how the pathogen changes and breakdown of resistant cultivars could take place. In France, *Rlm1* in French cultivars and in Australia *LepR3* genes in cultivar "Surpass400" are fine examples of resistance breakdown due to selection pressure exerted on the pathogen population by resistant cultivars (Li et al., 2003 and Rouxel et al., 2003). The appearance of new PG's in Western Canada and North Dakota may be related to selection pressure and sexual recombination (Fernando and Chen, 2003; Chen and Fernando, 2005; Bradley et al., 2005) or due to introduction of the new groups through seed. When Q-2 seed were grown in the greenhouse to maturity without any blackleg inoculations, there was significant blackleg-disease on the stem base (Ghanbarnia and Fernando, unpublished data). Analysis and monitoring of *Avr* gene structure and dynamics in the pathogen population will help us to achieve the goals in breeding for disease (blackleg) resistance. For example, *Rlm6*and *Rlm7* are probably resistant sources in European conditions due to the fact that *Avrlm6* and *Avrlm7* genes are prevalent in European isolates. However, those genes are limited in use in Australia and Canada as *Avrlm6* and *Avrlm7* genes are not found in these regions, or at least have not been reported so far. *Rlm1*, *Rlm2*, *Rlm3*, and *Rlm9* are still useful in Canada because *Avrlm1*, *Avrlm2*, *Avrlm3*, and *Avrlm9* alleles are detected in many Canadian isolates. However, those genes are fully overcome under European conditions (Balesdent et al., 2005).

Resistance to PG3 and PG4, new isolates found in Western Canada, should be mainly screened from worldwide collections of *B. rapa*. But it should be noticed that genetic control of avirulence to *B. rapa*was monogenic and the corresponding locus, termed *Avrlm8*, was genetically unlinked to any other *Avr* gene (Balesdent et al., 2002), which means that only single dominant resistance genes to PG3 and PG4 are expected.

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