

## **Detection of allelic diversity in resistance gene candidate sequences for association studies with blackleg disease in oilseed rape (*Brassica napus*)**

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### **Abstract**

Blackleg disease caused by the fungal pathogen *Leptoshaeria maculans* (anamorph *Phoma lingam*) is one of the most important oilseed rape (*Brassica napus*) diseases and can cause heavy yield loss of up to 50 % in epidemic years. A broad variation for *L. maculans* resistance is present in the *Brassica napus* gene pool, however due to the quantitative nature of the resistance it is difficult to develop effective selection markers. This project aims at detection and exploitation of genetic diversity of resistance gene candidates associated with blackleg resistance in *B. napus* via association studies between resistance data and single nucleotide polymorphism (SNP) haplotypes in a core set of 50 diverse oilseed rape genotypes. Resistance gene analogue (RGA) sequences have been amplified in resistant and non-resistant *B. napus* genotypes using degenerate primers for resistance gene motifs (TIR, NBS, LRR), and the resulting RGAs were cloned and sequenced. 48 unique RGAs were detected that showed the expected RGA sequence motifs. In addition to these anonymous RGA sequences, a further set of potential resistance gene candidates (RGCs) is being developed from 277 publicly available cDNAs derived from the *B. napus* defense reaction to inoculation with *L. maculans*. Sequence-specific primers were used to amplify and sequence the unique RGCs in eight genotypes showing a broad range of blackleg disease responses. Altogether we detected 82 SNPs and 16 InDels in RGAs and RGCs via sequencing, demonstrating the effectiveness of SNP markers for detection of allelic diversity. For all available sequences, sequence-independent SNP-detection is being performed with locus-specific primers in the entire core set, in order to construct haplotypes for association studies with *L. maculans* resistance/susceptibility data from field and greenhouse tests. Optional high-throughput procedures for SNP-detection are BessT/G (base excision sequence scanning) or EcoTILLING (targeting induced local lesions in genomes). SNP markers for haplotypes associated with quantitative resistance loci will be useful not only for marker-assisted selection, but also for identification of new resistance sources in the broader *B. napus* gene pool.