Identification of transcriptome-based molecular markers linked to stem-rust resistance in perennial ryegrass

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Perennial ryegrass (*Lolium perenne*) is one of the most important cool-season grass species in temperate zones worldwide and used in forage production and as turf grass. Seed production of ryegrass is affected by stem rust caused by the obligate biotrophic pathogen *Puccinia graminis* f.sp. *graminicola* and causes yield loss up to 98 %. A perennial ryegrass mapping population segregating for stem-rust resistance was screened with three stem rust field isolates in a leaf-segment test. Leaf segments of inoculated and non-inoculated resistant and susceptible individuals were bulked, respectively, at three time points (before inoculation, 4–8 and 18–24 hpi). Bulked segregant analysis of differential gene expression was accomplished via massive analysis of cDNA ends (MACE) and RNAseq. MACE detected genome-wide, quantitative expression profiles of 57 million transcripts along with their allelic diversity. Transcripts were assembled into 144,000 contigs and functionally annotated to the SwissProt database using BLASTX and the NCBI *Viridaeplantae* database using BLASTN. In total, 401 transcripts exclusively expressed in the "resistant" bulks (ERTs) were identified, including eight transcripts with homology to disease resistance genes. In addition, SNPs of RNAseq and MACE which occurred exclusively in the “resistant” bulks were filtered. ERTs and SNPs were annotated to the genome of the model grass species *Brachypodium distachyon*. Most of the ERTs and SNPs mapped on *Brachypodium* chromosome 1 (Bd1), with a peak falling into the physical region of 25.5 – 34.5 Mbp. To predict the genomic location of the stem-rust resistance gene in *L. perenne*, the perennial ryegrass GenomeZipper based on the conserved synteny between the grass species including *B. distachyon* was used. The peak of ERTs and SNPs on Bd1 showed macro-synteny to *L. perenne* chromosome 7. ERTs and SNPs annotated to Bd1 were used for PCR primer design. In total, 87 primer pairs were designed, of which 27 showed genetic linkage to stem-rust resistance.