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A transcriptome-based approach for developing breeding lines in *Lolium* sp. with multiple pathogen resistance

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Ryegrass (*Lolium* spec.) is the most important cool-season forage crop in temperate regions. Though, the seed production is considerably affected by several fungal and bacterial obligate biotrophic pathogens. The overall purpose of this study is directed to developing ryegrass cultivars with multiple pathogen resistance and agronomic adaption to Germany's agricultural conditions. This aim shall be achieved by combining genes for resistances to stem rust, crown rust and bacterial wilt. The pyramidisation shall be accomplished by the use of specific molecular markers which will be derived by bulked segregant analysis combined with next generation sequencing based massive analysis of cDNA ends (MACE) transcriptome profiling. RNA was isolated from bulks of infected and non-infected leaf segments from susceptible and resistant genotypes of various full-sibling mapping populations (n ≥ 200) and their respective parental lines for every investigated pathogen. After MACE was performed, bioinformatic analysis detects SNPs and transcripts that were exclusively expressed in the resistant bulk. Thus, 30 molecular markers were genetically mapped to a 50.8 cM spanning region surrounding the stem rust resistance locus *LpPg1*. The development of this high efficient molecular selection tool marks MACE as a fast and reliable method that detects polymorphisms for genetic mapping of candidate genes and obtains to be the method of choice for investigating the molecular and genetic base of resistances to stem rust, crown rust and bacterial wilt.