Pooled sequencing reveals multiple regions involved in resistance to bacterial wilt in Italian ryegrass

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Pooled sequencing reveals genome regions involved in resistance to bacterial wilt in Italian ryegrass

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Abstract. *Xanthomonas translucens* pv. *graminis* (Xtg) is the causal agent of bacterial wilt, one of the main diseases of Italian ryegrass (*Lolium multiflorum* Lam.). One major QTL for resistance was previously discovered, but the underlying genes are yet to be determined. In order to fine-map this QTL, a mapping population consisting of 7,484 F_2 individuals segregating for resistance was established in the greenhouse and inoculated with a highly virulent Xtg strain. Two pools of the most resistant and the most susceptible individuals were sequenced and SNPs associated with resistance were identified. Most of the significant SNPs map to linkage group 4, where the QTL was previously identified. Genes containing these SNPs will be determined and will constitute candidate resistance genes to be investigated further.

Keywords: Bacterial wilt, disease resistance, Italian ryegrass, *Lolium multiflorum* Lam., pooled sequencing, *Xanthomonas translucens* pv. *graminis*,

1 Introduction

Xanthomonas translucens pv. *graminis* (Xtg), the causal agent of bacterial wilt of forage grasses, is one of the most important pathogens of Italian ryegrass (*Lolium multiflorum* Lam.), causing serious yield and quality losses [1]. Cultivars with partial resistance to Xtg are available, however, since *L. multiflorum* is an allogamous species, cultivars have a high level of heterozygosity, and susceptibility still occurs in these cultivars. Marker-assisted selection would be greatly beneficial for selection and fixation of resistance alleles in *L. multiflorum* to breed resistant cultivars more rapidly and more efficiently.

Using an F_1 mapping population derived from a cross between a resistant and a susceptible parent (hereafter referred to as the Xtg-ART population), previous work identified a QTL explaining 43 to 84 % of resistance to Xtg on linkage group (LG) 4 [5]. However, further characterization of the QTL and the development of markers was hindered by the lack of sequence information for *L. multiflorum*

at the time. More recently, a pooled sequencing approach using the Xtg-ART population allowed the identification of SNPs associated to this QTL [2]. This served as a proof-of-concept for the pooled sequencing approach, but was limited in its resolution due to the low sequencing coverage and the small number of individuals used in the analysis.

We aimed at fine-mapping this QTL at high resolution by using a pooled sequencing approach in a large F_2 population derived from Xtg-ART using next-generation DNA sequencing technology.

2 Materials and Methods

A total of 7,484 F_2 individuals derived from Xtg-ART were established in the greenhouse, and leaves from each plant were harvested individually. Plants were allowed to grow back and were then inoculated with the highly pathogenic strain Xtg29 [4].

Disease was monitored and scored on a scale from one to five at 14, 21, 28, and 49 days post inoculation, and the 750 most resistant individuals and the 761 most susceptible individuals were selected to form a resistant and susceptible pool, respectively. Both pools were randomly divided into three subpopulations of an equal number of samples. DNA was extracted from the previously harvested leaves and libraries for each subpopulation were prepared for whole genome sequencing using Illumina TruSeq Nano DNA Library Preparation, (Illumina, San Francisco, CA, USA). Libraries were then sequenced using 150 bp paired-end reads on a NovaSeq 6000 S4 flowcell.

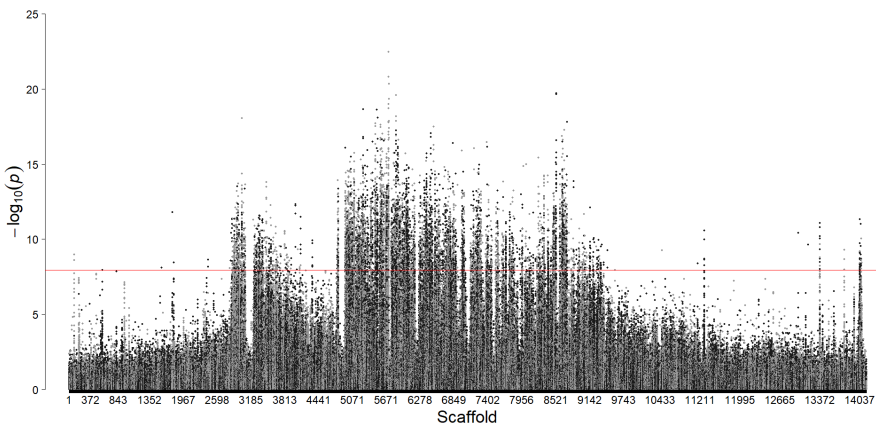

Fig. 1. Association between SNPs and resistance on linkage group 4. The X-axis shows each scaffold ordered based on its relative position on the genome sequence of barley (*Hordeum vulgare* L.). The Y-axis shows the -$\log_{10}$ of the p-value associated with each SNP. The red line indicates the significance threshold after Bonferroni correction.

Reads were mapped on the genome sequence of M2289, the resistant parent of the Xtg-ART population [3], and SNPs were determined using bcftools 'call' from a samtools 'mpileup'. After filtering for low (<50) and high (>400) read counts, and quality (>50 QUAL score), association between each SNP and resistance or susceptibility was tested by Cochran Mantel Haenszel test.

3 Results and Discussion

A total of 602,742 Gbp were obtained by sequencing (average = 100,457 Gbp per subpopulation), corresponding to an average of 44X coverage of the haploid genome per subpopulation. After mapping the reads to the M2289 genome sequence, 8,435,414 SNPs were identified, and 4,366,368 remained after filtering. The Cochran Mantel Haenszel test revealed 12,933 significant SNPs after Bonferroni correction. Most of these were located on LG 4, corresponding to the previously identified QTL (Fig. 1).

4 Conclusion

Using a pooled sequencing approach, we were able to identify SNPs associated with resistance to Xtg. Most of these SNPs are located on LG 4, where the QTL for resistance was previously identified, confirming the reliability of this approach. Moreover, the high number of progenies used, together with the sequencing coverage obtained, resulted in higher marker resolution compared to the previous QTL analyses, which were based on a small number of markers. Further analysis of the data will allow to identify candidate genes within this region, which will, after validation, allow to better understand the mechanisms of the interaction and to develop genomics-assisted breeding strategies to improve Xtg resistance in *L. multiflorum*.

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