Unravelling the Genetic Control of Bacterial Wilt Resistance in Ryegrass: Achievements, Prospects and Challenges

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Unravelling the genetic control of bacterial wilt resistance in ryegrass: achievements, prospects and challenges

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Abstract *Xanthomonas translucens* pv. *graminis* (*Xtg*) causes bacterial wilt, one of the most important forage grass diseases in temperate grasslands. Molecular genetic and genomic tools have the potential to significantly benefit resistance breeding and to enable targeted resistance management. In the past, a major QTL for bacterial wilt resistance was identified in *Lolium multiflorum* and *Xtg* was shown to rely on a non-canonical type III secretion system for plant infection. Recently, a number of candidate genes for bacterial wilt resistance were identified by comparing genomic sequences of resistant and susceptible parental plants and their progeny. Comparative genomics of different *X. translucens* pathovars allowed to identify virulence traits characteristic for *Xtg*. These candidate plant resistance genes together with the bacterial virulence factors provide an invaluable resource for the development of genomics assisted selection strategies. In addition, the well characterised plant genotypes and bacterial strains serve as an ideal model system to fully understand the complex *L. multiflorum*-*Xtg* interaction.

Keywords

Xanthomonas translucens pv. *graminis*, *Lolium multiflorum*, plant resistance, pathogen virulence

Introduction

Bacterial wilt, caused by *Xanthomonas translucens* pv. *graminis* (*Xtg*), is a devastating disease on forage grasses such as *Lolium multiflorum*, leading to significant yield and quality losses (Egli et al. 1975). Although cultivars with considerable resistance to bacterial wilt have been obtained through recurrent phenotypic selection (Suter et al. 2017), marker-assisted breeding would greatly benefit efficient selection and allow to fix dominant resistant genes in this allogamous, highly heterozygous species. Tremendous technical developments in the area of DNA and RNA sequencing (Goodwin et al. 2016), together with an evolving conceptual framework on hostpathogen interactions (Pritchard and Birch 2014) have opened new avenues for characterizing the genetic control of disease resistance in plants.

In this paper, we summarize past achievements towards understanding the complex interaction between *L. multiflorum* and *Xtg*, highlight current prospects for developing genomic breeding tools and touch on remaining challenges regarding breeding for bacterial wilt resistance in forage grasses.

Past achievements

Soon after the discovery of bacterial wilt, several pathovars of *X. translucens* have been distinguished based on their host specificity and selection for bacterial wilt resistance was soon integrated into breeding programs (Michel 2001). In a first attempt to characterize the genetic control of bacterial wilt resistance in *L. multiflorum*, Studer et al. (2006) identified a major quantitative trait locus (QTL) on linkage group (LG) 4 which explained up to 84 % of the phenotypic variance. However, marker-assisted introgression of this QTL in breeding programs was hindered by the lack of sequence specific markers in the QTL region. Although major QTL are often associated with race-specific major resistance genes, a screening of 62 plant genotypes revealed no major race-specific interactions between *L. multiflorum* and *Xtg* (Wichmann et al. 2011b). Further evidence for broad-spectrum rather than race-specific resistance was gained from transcriptome analyses, where transcriptional changes usually triggered by pathogen associated molecular patterns were observed (Wichmann et al. 2011a).

However, whole genome sequencing of *Xtg* revealed the existence of a non-canonical but functional type III secretions system (T3SS; Wichmann et al. 2013). The T3SS is crucial for effector translocation from the pathogen to the host in effector-triggered immunity (Pritchard and Birch 2014). In *Xtg*, T3SS mutants have been

shown to lose their ability to cause disease in *L. multiflorum* but to keep their ability for *in planta* multiplication (Wichmann et al. 2013). Another characteristic of *Xtg* strains was their relatively low genetic diversity when analysed across a larger geographic range using amplified fragment length polymorphism markers (Kölliker et al. 2006).

Current prospects

In order to generate a resource for sequence-based resistance gene discovery in *L. multiflorum* , and to further characterise the QTL on LG4, the resistant and the susceptible parental plants of the *Xtg* mapping population were sequenced using Illumina HiSeq, resulting in a total of $18x10^6$ heterozygous single nucleotide polymorphisms (SNPs) between the two parents (Knorst et al. 2016). In addition, genomic sequences of DNA pools of the 57 most resistant and the 50 most susceptible F_1 individuals were produced, aligned to the parental reference sequence and SNPs between the resistant and susceptible DNA pools were identified.

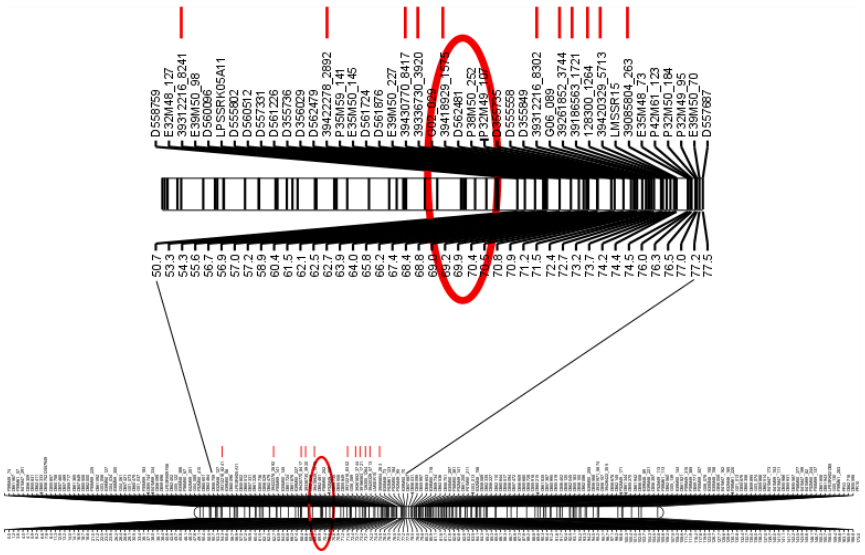

Fig. 1. Linkage group 4 of the *L. multiflorum* linkage map (improved based on Studer et al. 2006) with locations of the QTL for bacterial wilt resistance (circle) and 11 candidate scaffolds (bars) identified through genome sequencing of parental genotypes and DNA pools of resistant and susceptible progeny, respectively.

Scaffolds containing such SNPs were aligned with the *L. perenne* genome sequence (Byrne et al. 2015). Candidate scaffolds allocated to LG 4 were mapped to the *L. multiflorum* linkage map using SNP data from KASP assays (LGC, Middlesex, UK). The eleven candidate scaffolds mapped to the region where the initial QTL for bacterial wilt resistance was identified, spanning a region from 54 to 74 cM on LG 4 (Fig. 1). The identified candidate scaffolds not only mapped in the vicinity of the QTL, they also showed homologies to genes known to be involved in disease resistance such as for example serine/threonine kinases and therefore present a valuable resource for targeted breeding of bacterial wilt resistance.

Detailed characterisation of bacterial virulence may not only allow to better understand complex host-pathogen interactions, but also to identify and exploit plant resistant traits which correspond to bacterial virulence factors.

Table 1 Average nucleotide identities of *X. translucens* pathovars pathogenic (*Xtg, Xta, Xtp*) and non-pathogenic (*Xtt, Xtc)* on forage grasses.

	Xtg29	*Xtg2*	*Xtg9*	*Xtg10*	*Xta*	*Xtp*	*Xtt*
Xtg2	**99.94**						
Xtg9	**99.93**	**99.92**					
Xtg10	**99.92**	**99.93**	**99.95**				
Xta	97.98	97.96	97.96	97.95			
Xtp	97.67	97.6	97.62	97.61	98.00		
Xtt	*95.98*	*95.98*	*95.98*	*95.94*	*95.96*	*95.91*	
Xtc	*95.35*	*95.35*	*95.33*	*95.33*	*95.42*	*95.31*	*95.34*

Data represent the mean percentage of identity of orthologues shared by the strains. *Xtg: X. translucens* pv. *graminis* strains from Switzerland, *Xta: X. translucens* pv. *arrhenateri* (LMG 727), *Xtp: X. translucens* pv. *poae* (LMG 727), *Xtt*: *X. translucens* pv. *translucens* (DSM 18974), *Xtc: X. translucens* pv. *cerealis* (CFBP 2541). Modified from Hersemann et al. (2017).

Comparative genomic analyses of various *X. translucens* pathovars revealed very high nucleotide identity among *Xtg* strains (> 99 %), high nucleotide identity among

Xtg and other forage grass infecting pathovars (> 97 %) and only moderate identity among strains pathogenic and non-pathogenic on forage grasses (< 96 %; Table 1). When comparing the *Xtg* core genome to non-*graminis X. translucens* strains, a set of 74 coding sequences (CDS) were found to be unique to *Xtg*. Thirty of these were functionally annotated and were assigned to four categories, i.e. nutrient acquisition, regulation & modification, virulence and adhesion & motility (Hersemann et al. 2017). These unique CDS may play an important role in the plant-pathogen interaction. In addition, when comparing surface exposed structures such as components of the T3SS or the type IV pilus, high sequence deviations were found for *Xtg* when compared to the other *X. translucens* strains. This may allow *Xtg* to evade plant perception and enable successful colonization of *L. multiflorum* (Hersemann et al. 2017).

Remaining challenges

The candidate scaffolds for bacterial wilt resistance identified in *L. multiflorum*, together with the bacterial virulence traits identified in *Xtg* represent with no doubt an invaluable resource for the development of genome assisted selection strategies for bacterial wilt resistance. However, in order to fully understand plant resistance, which seems to be controlled by complex general regulating mechanisms rather than effector triggered immunity responses, the interplay of bacterial virulence traits and plant resistance mechanisms needs to be further investigated. In this context, dual RNA-seq may offer a valuable tool to simultaneously investigate host and pathogen transcriptomes (Westermann et al. 2016). Another potential limitation lies in the phenotypic characterisation of the host-pathogen interaction. So far, all studies relied on scoring of disease symptoms (wilting) on clonally propagated plants. Not only does this method often yield variable data within the same genotype (unpublished data), it most likely also lacks the sensitivity needed to detect subtle changes in the early stages of pathogen infection.

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