FINE MAPPING OF A POWDERY MILDEW QTL BY EXOME-SEQUENCING

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Fine mapping
PAV **Powdery mildew**
Exome capture
NBS-LRR genes **Barley**
Sequencing
Disease resistance

INTRODUCTION

Powdery mildew (*Blumeria graminis*) is a fungal ectoparasite which leads to severe losses in yield and grain quality in barley. In this work, we took advantage of state-of-the-art genomic tools available for barley to **fine map a resistance QTL** contributed by a Spanish landrace (Silvar et al. 2010). A large **F2 population** was created and screened with genetic markers. **Phenotyping** revealed 3 recombinant lines with different resistance/susceptibility scores in an narrow genetic interval. **Exome sequencing** of those lines and the parents allowed to locate the physical position of the resistance gene, which led to find a cluster of **NBS-LRR genes** with PAV, among which one candidate gene was identified.

METHODOLOGY

Plant material, mapping population and genotyping

A BC_1F_2 population was obtained from the cross Plaisant x (RIL151 (SBCC097 x Plaisant)). Genotyping was carried out with CAPS markers and one SSR. 20 BC_1F_3 lines of each selected BC_1F_2 recombinant plant were screened to obtain homozygous BC_1F_4 recombinant lines.

Disease assessment

Four isolates of *B. graminis* f. sp. *hordei* were used to score resistance/susceptibility in the parents and BC_1F_4 recombinant lines. Five plants per line were inoculated. Infection types were recorded on a scale of 0 to 4, 10d after inoculation.

Exome sequencing

Genomic DNA was extracted from leaf tissue, captured with exome-targeted baits (Mascher et al. 2013) and sequenced in an Illumina HiSeq2000. Variant detection was performed by alignment to the Morex WGS assembly (IBGSC 2012). BACs in the region were obtained from both IBGSC and UCR (Muñoz-Amatriaín et al. 2015) assemblies. Gene annotation was obtained by alignment to UniprotKB and enriched after specifically searching for NBS and LRR motifs. Regions with PAV genes (see **Figure 1**) were located by k-mer analysis. Reads mapping to those regions were de-novo assembled.

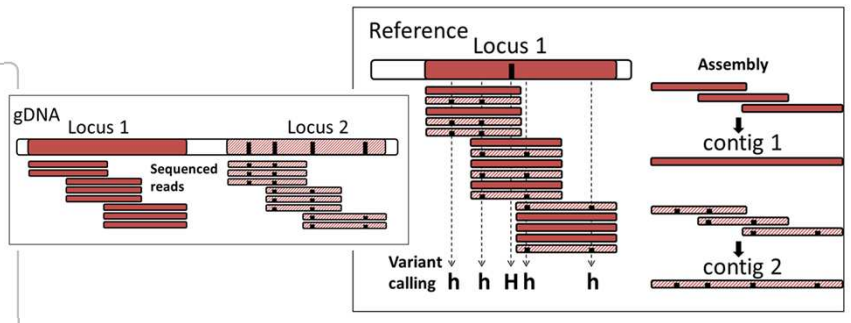


Figure 1 – Example of assembly of genes absent in the reference. Left: reads from two paralogous loci are sequenced. Right: after mapping to the reference, lacking one locus, heterozygous variant callings ("h") are produced, besides homozygous callings ("H"). Reads from those mappings are assembled, which could lead to independent contigs ressembling the loci which were source of the reads. By annotating the recombinant line in each contig and performing sequence clustering, PAV genes could be genotyped to identify those present or absent at given genotypes.

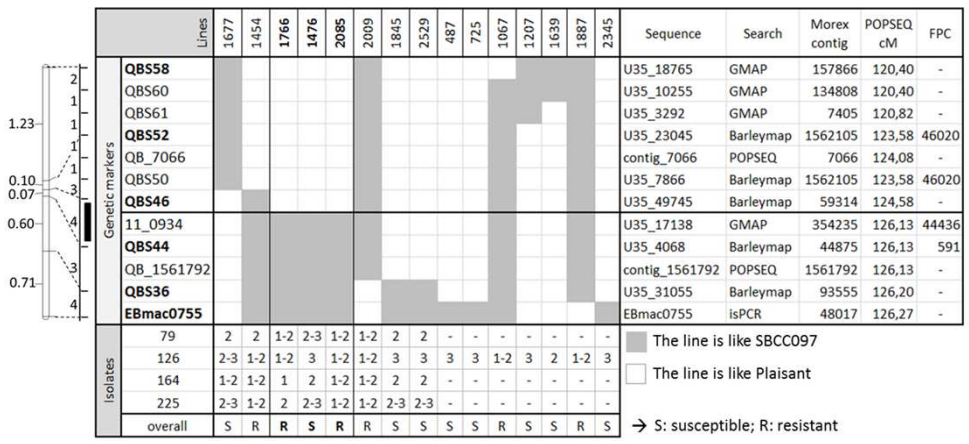


Figure 2 – Genotyping and phenotyping of the 15 BC_1F_4 homozygous recombinant lines. An horizontal line and two vertical lines highlight the markers and lines which point towards the genetical position of the resistance locus. Columns to the right show POPSEQ positions of the genetic markers, obtained by combining Barleymap (Cantalapiedra et al. 2015), GMAC chimeras and isPCR.

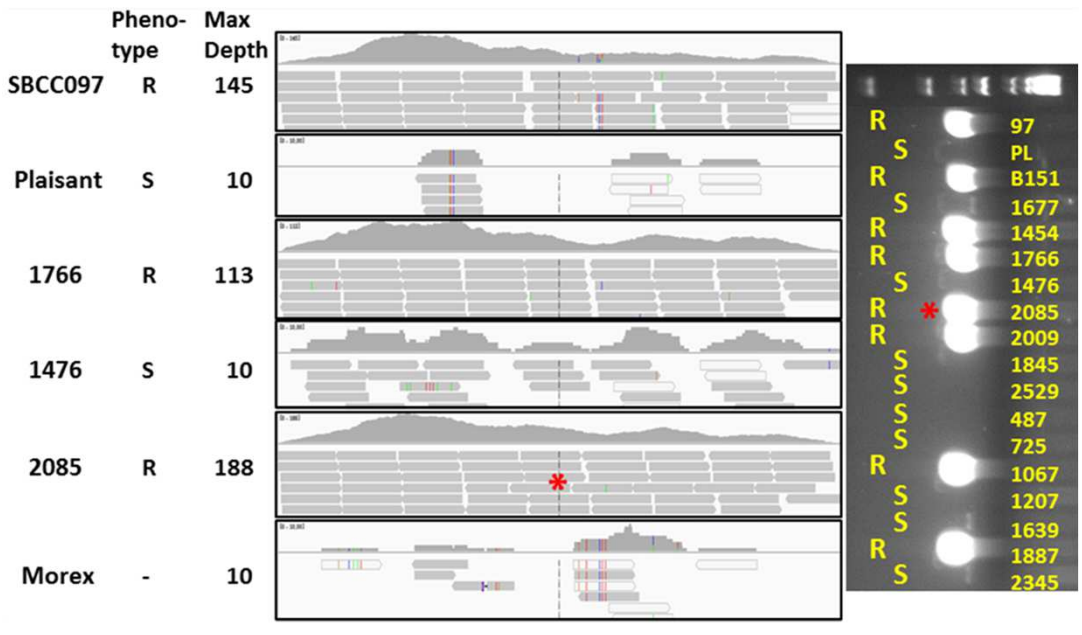


Figure 4 – Genotyping of the newly assembled NBS-LRR gene (**ELOC1**). Left: read mapping of exome sequencing data to the new contig, including the two parents, the three BC_1F_4 lines and cultivar Morex as control. Right: PCR amplification products of the new contig, for the two parents, the RIL151 carrying the SBCC097 QTL at 7HL (derived from SBCC097xPlaisant) and the 15 BC_1F_4 homozygous recombinant lines. R: resistant line; S: susceptible line.

RESULTS

Fine mapping of the resistance locus

Out of 2899 BC_1F_2 plants tested, 152 BC_1F_3 recombinants were identified and further tested, leading to 15 BC_1F_4 homozygous recombinants covering the QTL region (**Figure 2**). A genetic map of the region revealed a 0.07 cM interval between closest markers. Three BC_1F_4 lines, one susceptible and two resistant, had the same genotype flanking the markers in such interval.

Physical localization of the resistance locus

Morex WGS contigs in the region were obtained mapping genetic markers to the POPSEQ map. Scoring of variants in the region allowed identifying FPC591 as the likely phisical location of the resistance gene (**Figure 3**).

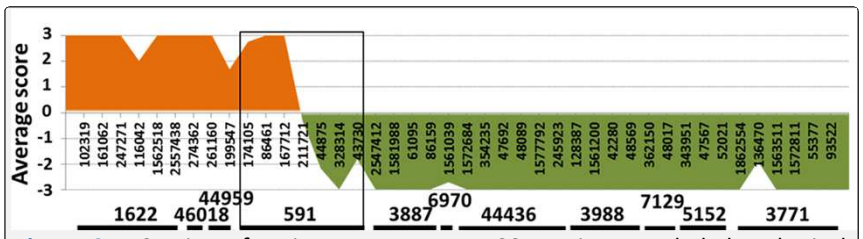
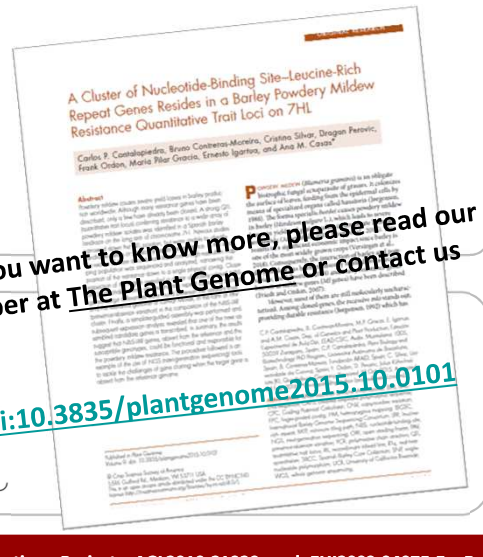


Figure 3 – Scoring of variants on Morex WGS contigs revealed the physical position of the resistance gene. Orange: genotypes are like Plaisant. Green: genotypes are like SBCC097.

Searching for candidate genes

By combining IBGSC and UCR assemblies, we were able to obtain BAC contigs covering the whole resistance locus. However, assemblies of those BACs were highly fragmented. Nevertheless, a cluster of several closely related genes coding for NBS-LRR proteins were identified. Analysis of heterozygous variants in the region, and a highly sensitive de-novo assembly (**Figure 1**), allowed to identify genes from the same protein family, showing PAV in the resistant and susceptible genotypes. One of those sequences (**ELOC1**) was present only in the resistant BC_1F_4 lines, which was confirmed through read mapping and by PCR amplification (**Figure 4**).

RT-qPCR revealed positive amplification of **ELOC1** transcripts in leaves from SBCC097 and the two resistant BC_1F_4 lines. However, analysis of its expression at different time points after infection did not reveal changes in transcript levels.



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doi:10.3835/plantgenome2015.10.0101

COMMENTS AND FURTHER WORK

The present work allowed narrowing down the position of a resistance QTL to a 0.07 cM genetic interval. Exome sequencing pinpointed the physical location of the responsible gene and revealed a cluster of NBS-LRR genes with PAV, as potential candidate genes. The new Morex genome assembly will allow to further inspect the region. However, sequencing of BACs from SBCC097 could be required if the gene responsible of the resistance to powdery mildew is actually absent from the reference genotype.

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ACKNOWLEDGEMENTS: work funded by the Spanish Ministry of Science and Innovation, Projects AGL2010-21929 and EUI2009-04075-ExpResBar, AGL2013-48756R, RFP2012-00015-00-00, and by the German Federal Ministry of Education and Research, grant number 0315702B within the KBBE-II call. CP Cantalapiedra was supported by an FPI scholarship.