INTRODUCTION

Powdery mildew (Blumeria graminis) is a fungal ecto-parasite 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. Exome sequencing 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 BC4 population was obtained from the cross Plaisant x SBCC097. Genotyping was carried out with CAPS markers and one SSR. 20 BC4 lines of each selected BC4 recombinant plant were screened to obtain homozygous BC4 recombinant lines. Disease assessment
Four isolates of B. graminis f. sp. hordei were used to score resistance/susceptibility in the parents and BC4 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 (Masher et al. 2013) and sequenced in an Illumina HiSeq2000. Variant detection was performed by alignment to the Morex WGS assembly (IBSC 2012). BACs in the region were obtained from both IBGSC and UCR (Muñoz-Amatriain 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.

RESULTS

Fine mapping of the resistance locus
Out of 2899 BC4 F2 plants tested, 152 BC4 recombinants were identified and further tested, leading to 15 BC4 homozygous recombinants covering the QTL region (Figure 2). A genetic map of the region revealed a 0.07 cM interval between closest markers. Three BC4 F2 lines, one susceptible and two resistant, had the same genotype flanking the 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 FPC091 as the likely physical location of the resistance gene (Figure 3).

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 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 4), 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 BC4 F2 lines, which was confirmed through read mapping and by PCR amplification (Figure 4).

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 SBC097 could be required if the gene responsible of the resistance to powdery mildew is actually absent from the reference genotype.

REFERENCES

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