Research of candidate genes in peach for resistance to root-knot nematodes and resistance to aphids Myzus persicae

Henri Duval, Laure Heurtevin, Naïma Dlalah, Jacques Lagnel, Caroline Callot

Research of candidate genes in peach for resistance to root-knot nematodes and resistance to aphids *Myzus persicae*

H. Duval[1], L. Heurtevin[1], N. Dlalah[1], J. Lagnel[1] and C. Callot[2]

[1] INRAE, GAFL, 84143, Montfavet, France

[2] CNRGV, INRAE, CNRS, Castanet Tolosan, France.

INTRODUCTION

One objective of the INRAe peach rootstock breeding program is to accumulate the Rmia gene of resistance to root-knot nematodes and the Rm1 gene of resistance to peach green aphid, Although we are using markers that are already available (Duval et al., 2014) (Lambert et al., 2016), they cannot be used in some progeny to screen for resistant hybrids, particularly in interspecific progeny such as peach*almond crosses. It was therefore necessary to continue research until the genes were identified. The sequencing of the peach genome, combined with the availability of new sequencing technologies (NGS, RNAseq, Pacbio), provided powerful tools for detailed molecular studies and the identification of new genes. In the INRAe germplasm collection, the peach clone "Weeping Flower Peach" (WFP, clone S2678) was introduced in 1961 from Clemson University (Clemson, SC) with unknown origin. It has double flowers, green leaves, weeping growth habit and is interesting for its resistance to aphids,(*Myzus persicae*) and root-knot nematodes (Meloidogyne incognita and arenaria) such as Nemared peach rootstock.

The objectives of our study were to obtain the complete sequence and transcripts of the "WFP" clone, and to identify resistant candidate genes in the region targeted on group 1 for the RM1 gene and on group 2 for the Rmia gene.

MATERIEL AND METHODS

NGS sequencing of the WFP, Rubira, Nemared peach varieties was carried out at the MGX-Montpellier platform, which performed sequencing a paired-end 125 using an Illumina Hiseq 2500. We then built a bacterial artificial chromosome (BAC) library at the CNRGV in Toulouse. The resulting library represents a 7.60 x coverage of P. persica cv. WFP with 18432 BAC clones and an average insert size of 119 kb. The BAC clones were located on a nylon membrane, screened with labelled radioactive probes and revealed by a high-density filter reading programme. BAC clones were spotted on nylon membrane, screened with radioactive labelled probes and revealed by High-density filter reader program. Some probes were designed after the WFP mapping of the NGS reads on the peach V2 genome, in order to screen Bac clones containing the target regions for the aphid and nematode resistance genes. We screened 27 BAC clones for the Rm1 region and 11 BAC clones for the Rmia region. We selected five BAC clones for sequencing them using the PacBio long-read sequencing technology to overcome potential assembly errors due to repeated sequences. Finally, we extracted RNA from the apex leaves and roots and built a full- Length RNA-Seq Library and we used the PacBio Sequel® Systems to sequence transcriptomes.

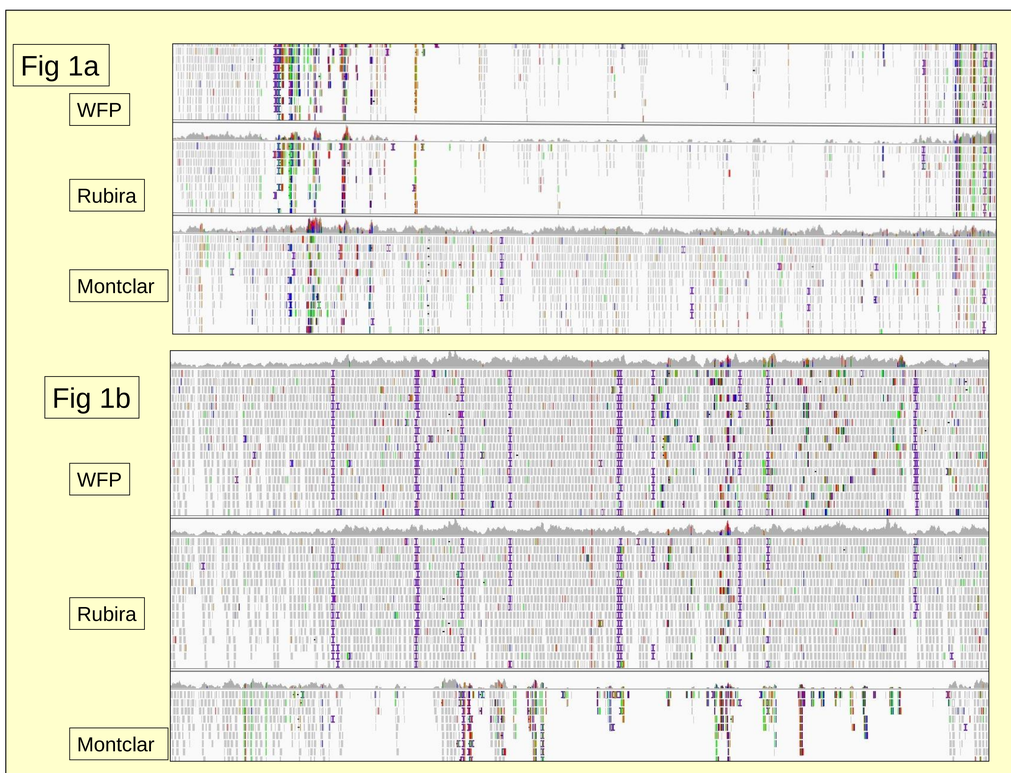


Figure 1: Alignments of NGS reads of WFP, Rubira and Montclar
a) On the reference Peach v2.0 in the Rm1 region. (group 1)
b) On the sequence of the screened Rm1 BAC clone of WFP

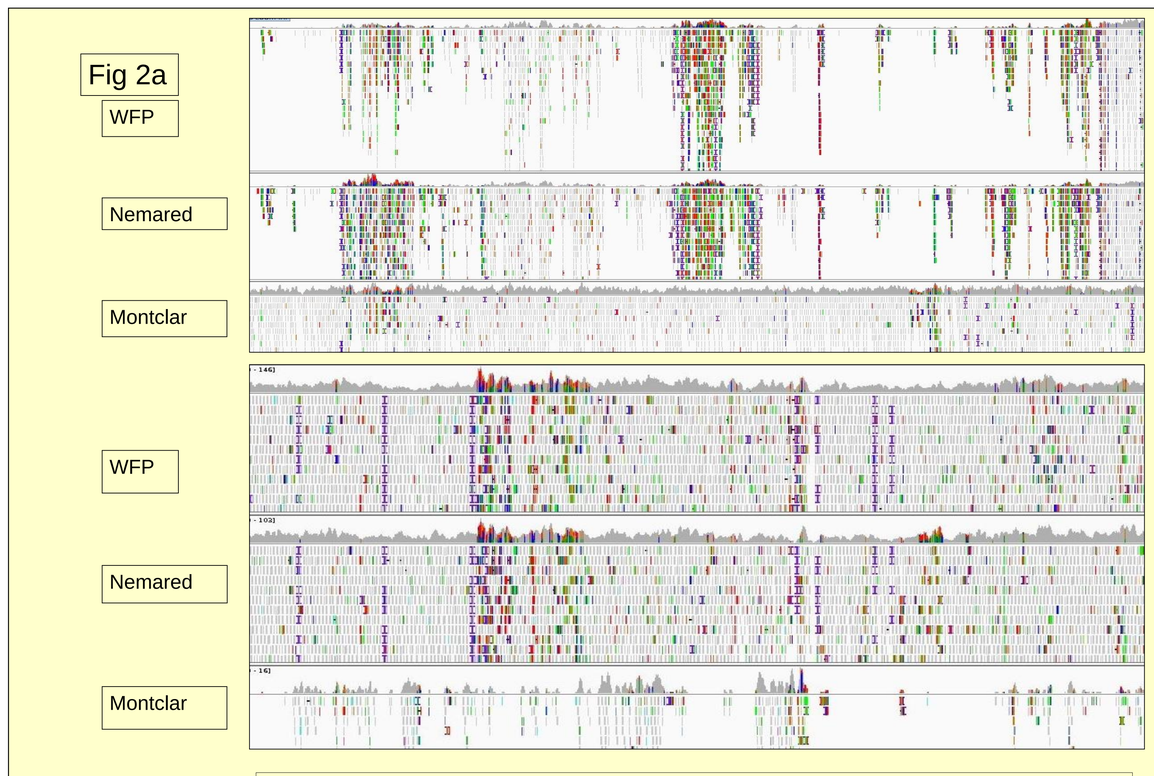


Figure 2: Alignments of NGS reads of WFP, Nemared and Montclar
a) On the reference Peach v2.0 in the Rmia region. (group 2)
b) On the sequence of the screened Rmia BAC clone of WFP

RESULTS /

The IGV software allowed seeing that the alignments of the WFP, Rubira, Nemared and Montclar short-reads with the reference Peach v2.0.a1 or with the new sequences of the new BACs clones. The figure 1a shows the alignment in a part of the Rm1 region in the range from 45.665Kb to 46.120Kb of the Peach V2 group1. Alignments of WFP and Rubira were not suitable compared to those of the sensitive Montclar. When we performed a new alignment on the new sequence of the Rm1 BAC clones, we obtained an accurate mapping of the WFP and Rubira reads (Fig 1b) in contrast to the Montclar reads. We can conclude that the Rm1 region sequence is very different from those of the Lovel peach reference. We also could see that both aphid-resistant WFP and Rubira had the same DNA read profiles and that both varieties probably have the same aphid-resistant gene.

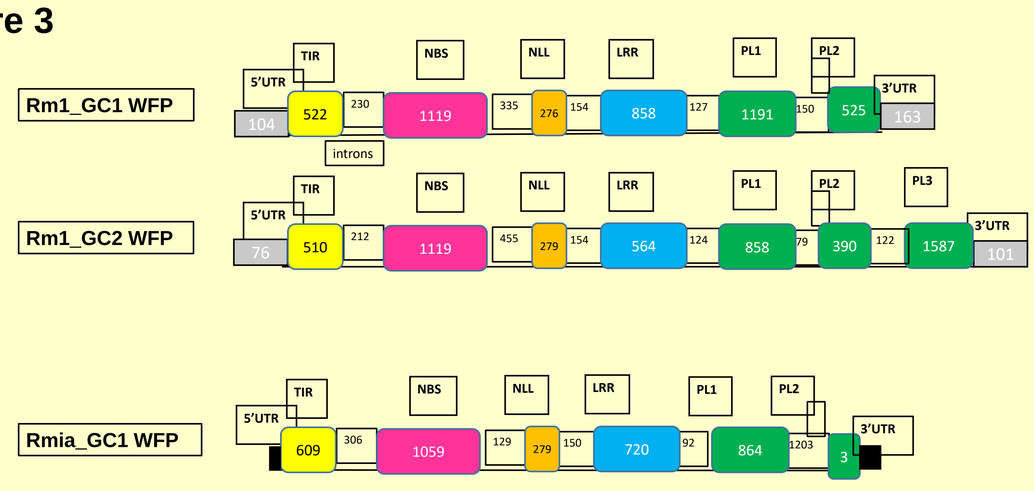
As with Rm1, Figure 2a shows the alignment in part of the Rmia region in the range from 6.574Kb to 6.696Kb of the group 2 of Peach V2. The alignments of WFP and Nemared were not good compared to those of the sensitive Montclar. When we proceeded a new alignment on the new sequence of Rmia BAC clones, we obtained a good mapping of the WFP and Nemared NGS reads (Fig 2b) in contrast to the Montclar reads. We can conclude that the Rmia region sequence is very different from that of the Lovel peach reference and we also found that the two nematode resistant WFP and Nemared have the same DNA reads profiles and that both varieties probably have the same nematode resistance gene.

LONG-READ RNA-SEQ ANALYSIS

From the alignment of the WFP RNAseq long-reads on the Rm1bac region, we obtained a Bed file where thirty-one genes were expressed. Four new genes were annotated as plant-defence genes, but only two of them were expressed in the resistant genotypes and not in the sensitive genotypes. For the alignment of WFP RNAseq long-reads on the Rmia bac region, we observed the expression of only one new gene. For these three new candidate genes (Rm1_CG1, Rm1_CG2, Rmia_CG1), the exons, introns, start codons and codons stop were located from the transcript sequences and the protein translation could be determined (Figure 3).

The three proteins have respectively 1497, 1769 and 1178 amino acids. The analyse of the structure of these proteins, performed with Interproscan, shows that the three genes belong to the family of TIR-NBS-LRR (TNL) genes with the presence of a Toll-interleukin-1 receptor domain in the N-terminal part of the protein and a complete structure with full-length domains,

Figure 3



References :

- Duval, H., Hoerter, M., Polidori, J., Confolent, C., Masse, M., Moretti, A., Van Ghelder, C., Esmenjaud, D., 2014. High-resolution mapping of the RMia gene for resistance to root-knot nematodes in peach. Tree Genet. Genomes 10, 297–306. https://doi.org/10.1007/s11295-013-0683-z
- Lambert, P., Campoy, J.A., Pacheco, I., Mauroux, J.-B., Linge, C.D.S., Micheletti, D., Bassi, D., Rossini, L., Dirlewanger, E., Pascal, T., Troggio, M., Jose Aranzana, M., Patocchi, A., Arus, P., 2016. Identifying SNP markers tightly associated with six major genes in peach [Prunus persica (L.) Batsch] using a high-density SNP array with an objective of marker-assisted selection (MAS). Tree Genet. Genomes 12, 121. https://doi.org/10.1007/s11295-016-1080-1