Identification of QTLs for *Fom* race 1.2 resistance using a genotyping by sequencing approach

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INTRODUCTION

Fusarium wilt of melon (*Cucumis melon* L.), caused by *Fusarium oxysporum* Schlecht. f. sp. *melonis* Snyd. & Hans. (*Fom*) is an important disease spread worldwide. Race 1.2, subdivided into pathotype 1.2y (yellow) and 1.2w (wilt), overcomes all resistance genes described and could become a serious threat to this crop cultivation. The development of cultivars exhibiting enhanced resistance to *Fom* race 1.2 is an important objective of breeding programs. The objective of our study was the identification of QTLs linked to the resistance to *Fom* race 1.2.

MATERIAL AND METHODS

Partial resistance to *Fom* race 1.2 was studied using a total of 117 recombinant inbred lines (RILs) derived from a cross between the susceptible cultivar 'Piel de Sapo', a highly valuable cultivar, and 'BG-5384', a partially resistant line (Chikh-Rouhou et al. 2007). Artificial inoculations were performed with the *Fom* isolate 37mls belonging to the pathotype 1.2w and *Fom* isolate 24ml, belonging to the pathotype 1.2y. Inoculations were carried out in two environments, growth chamber and greenhouse. Disease severity was assessed using the following scale: 1= no symptoms; 2= beginning of wilting or yellowing on leaves; 3 =leaves heavily affected by wilting or yellowing; 4 = all leaves completely wilted, stem standing; and 5 = dead plant. For the statistical analyses, the values of the area under the disease progress curve (AUDPC) were used (Perchepied and Pitrat, 2004). Phenotypic data were analyzed in GenStat 17th Ed (VSN Internacional Ltda). Genotyping was carried out by using genotyping-by-sequencing (GBS) approach by Cornell University Biotechnology Resource Center (NY, USA). Association analysis was carried out using Tassel 5.0 (Bradbury et al. 2007). A general linear model (GLM) and mixed linear models (MLM) were selected to test marker trait associations.

RESULTS AND DISCUSSION

The AUDPC means for pathotype 1.2y were in the range of 1,1- 36,7 in chamber and 0,0-42,0 in greenhouse. For pathotype 1.2w, the values were in the range 0,6-44,0 and 0,0-43,0 for chamber and greenhouse, respectively (Fig. 1). Differences in AUDPC between environments were highly significant. However, the pathotype effect was not significant. There was also highly significant pathotype x environment interaction. Such interaction was also observed by Oumouloud et al (2013).

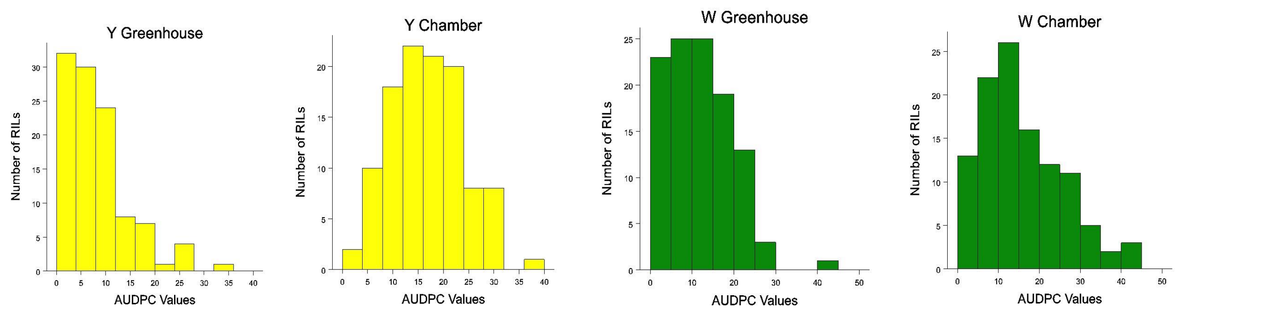

Fig. 1 Distribution of RILs according AUDPC for pathotype (1.2w and 1.2 y) and environments (greenhouse and chamber)

Genotyping was carried out by using GBS approach. After strict filtering of SNPs on the basis of read depth and minor allele frequency (MAF > 0.05), a final set of 2,625 SNPS was obtained and used for association analysis. Markers were plotted to their respective chromosomes to visualize their real position and total coverage (Fig. 2).

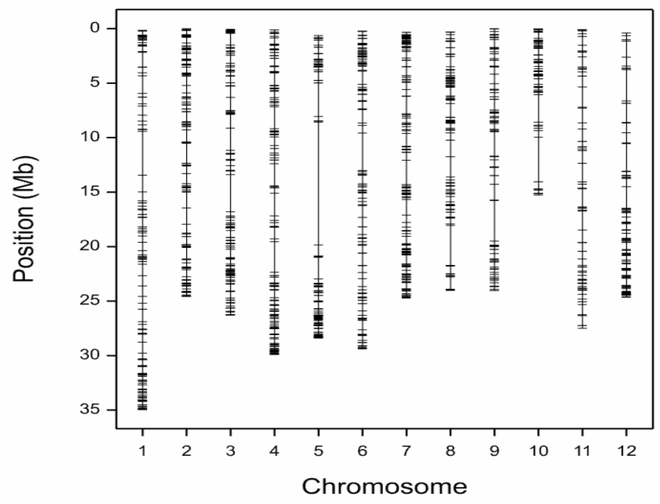

Fig. 2 Diagram of distribution of the preprocessed SNPs set for pathotype 1.2y_ greenhouse over all 12 chromosomes.

Association analysis revealed polygenic control with six chromosomal regions harboring significant marker trait associations (Fig. 3). The 6 SNPs markers located on chromosomes 1, 2, 6, 9, 10, and 11 (Table 1). Those showed to be environment-specific and pathotype-specific markers. Partial resistance to *Fom* 1.2 governed by a strain specific locus was also found by Perchepied et al. (2005).
Previous QTLs for *Fom*1.2 resistance have been located in linkage group (LG) 2 (Herman et al 2008), in LG6 and LG11 (Perchepied et al. 2005). In this work, new QTLs associated with resistance to *Fom* race 1.2 have been detected in LG1, LG9 and LG10.

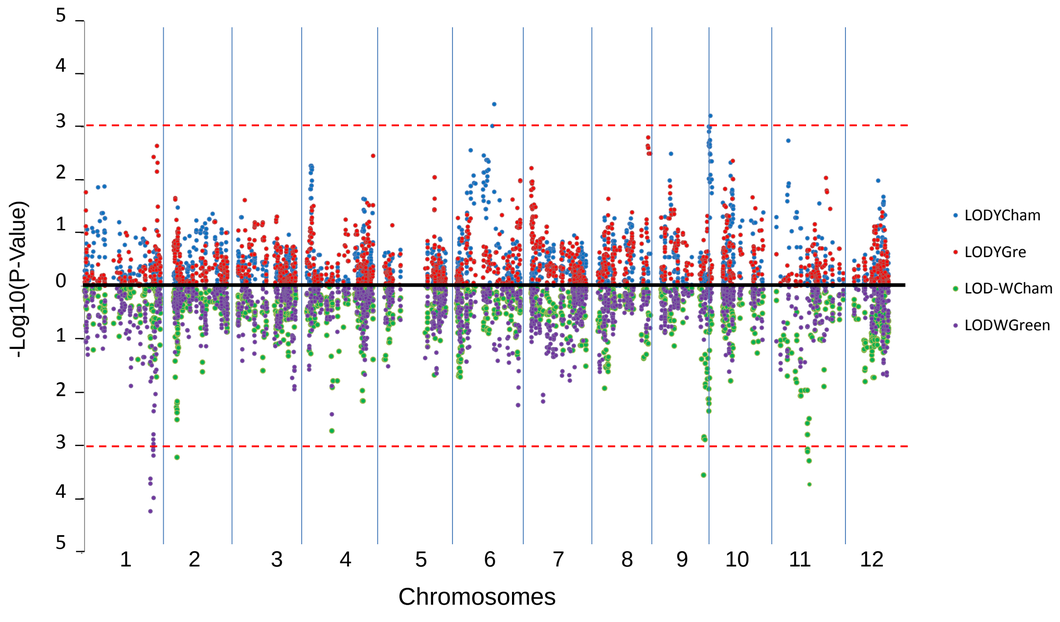

Fig. 3 Association scan for AUDPC for pathotypes 1.2y and 1.2w in two environments (chamber and greenhouse). The vertical axis plots the –log10(P) values of the association between the markers and disease severity (AUDPC).

Pathotype	Environment	Chr.	SNP position (bp)	LOD Score	Genetic Variance	Mean effect
Fom1.2w	Growth Chamber	2	1591748	3.23	13.44	6.84
Fom1.2w	Growth Chamber	9	21329237	3.57	36.33	-9.08
Fom1.2w	Growth Chamber	11	22247246	3.74	5.10	-8.46
Fom1.2w	Greenhouse	1	30344248	4.25	4.70	-6.03
Fom1.2y	Growth Chamber	6	19894145	3.43	3.25	4.99
Fom1.2y	Growth Chamber	10	1073191	3.21	3.20	4.97

Table 1. SNP markers associated with disease severity (AUDPC) for pathotypes (1.2w and 1.2y) and in two environments (greenhouse and chamber).

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