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## **QTL analysis reveals new eggplant loci involved in resistance to fungal wilts**

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## **Abstract (max 250)**

*Fusarium* spp. and *Verticillium* spp. are widespread soil pathogens responsible for vascular wilts causing heavy yield losses in eggplant (*Solanum melongena*) as well as in many other crops. Here we report on the identification of QTLs affecting the resistance to *Fusarium* and *Verticillium* in an F<sub>2</sub> intraspecific population of 156 individuals bred from the cross '305E40' x '67/3', we previously characterized for key agronomic and biochemical traits. The female parent ('305E40') is an androgenetic introgressed line carrying the resistance locus *Rfo-Sa1* derived from *Solanum aethiopicum*. The line is fully resistant to *Fusarium* and also displays a previously uncharacterized partial resistance to *Verticillium*. The male parent ('67/3') is an F<sub>8</sub> selection from the eggplant intra-specific cross cv. 'Purpura' x cv. 'CIN2' which, unexpectedly, revealed a not previously characterized partial resistance to *Fusarium*, but it is highly susceptible to *Verticillium*. The degree of resistance of the F<sub>2</sub> population was assessed following artificial inoculation in greenhouse (*Fusarium*) or growth chamber (*Verticillium*) of F<sub>2.3</sub> progenies obtained by selfing each F<sub>2</sub> individual. Other than a major QTL for the resistance to *Fusarium*, which lies in the genomic region of the *Rfo-Sa1* locus, major and minor QTL influencing the response to both *Fusarium* and *Verticillium* were spotted, and putative tomato orthologous genes were identified as well. The markers linked to the spotted QTL may find application in the context of marker-assisted breeding.

## **Keywords**

*Solanum melongena*, *Fusarium oxysporum*, *Verticillium* spp, QTL, biotic stress, resistance genes

## Introduction

Eggplant, also known as brinjal or aubergine (*Solanum melongena* L.,  $2n=2x=24$ ) is a member of the *Solanaceae*, a large plant family including around 2,700 plant species among which tomato (*S. lycopersicum* L.), potato (*S. tuberosum* L.), pepper (*Capsicum annuum* L.) and tobacco (*Nicotiana tabacum* L.). Unlike most of the other major Solanaceous crops, which are native of the New World (Fukuoka et al. 2010; Albert and Chang 2014; Hirakawa et al. 2014), eggplant has a uniqueness phylogeny due to its Old World domestication (Lester and Hasan 1991), which occurred in Asia as a result of at least two separate events (Daunay 2012; Meyer et al. 2012; Cericola et al. 2013, Knapp et al. 2013).

Eggplant is cultivated worldwide, with a total production of more than 50.2M tons in 2014, however, more than 90% of its production is concentrated in Asia, where it represents a staple food in countries such as China and India (FAOSTAT 2014, <http://faostat3.fao.org/browse/Q/QC/E>). Because of its importance for food security, eggplant is included, with 34 other crops, in the Annex 1 of the International Treaty on Plant Genetic Resources for Food and Agriculture (Fowler et al. 2003).

Eggplant is susceptible to many diseases, which cause yield losses both in greenhouse and in open field cultivations (Sihachakr et al. 1994), and the fungal wilts caused by *Verticillium dahliae* (*Vd*) Kleb., *V. albo-atrum* (McKeen 1972; Bhat et al. 1999; Diwan et al. 1999; Karagiannidis et al. 2002;) as well as *Fusarium oxysporum* f. sp. *melongenae* (*Fom*) (Stravato et al. 1993; Urrutia Herrada et al. 2004, Altinok et al. 2005) are among the most common and serious. As occurs in many other domesticates, anthropogenic selection has caused a drastic reduction of the genetic variation in cultivated germplasm, thus hampering the identification of resistance traits and their exploitation in breeding programs (Daunay et al. 1991, Rotino et al. 2014). On the other hand, the other two inter-fertile eggplant cultivated species, i.e. *S. aethiopicum* L. and *S. macrocarpon* L., as well as wild and allied relatives which share a relatively recent common ancestry with eggplant, represent a reservoirs of potential useful resistance traits exploitable for breeding purposes (Plazas et al. 2016, Syfert et al. 2016). Wild and cultivated relatives have been employed through both conventional (sexual crosses) and un-conventional (protoplast fusion, embryo rescue) strategies for the introgression of resistance traits in cultivated eggplant (Rotino et al. 2014; Kaushik et al. 2016), such as partial resistance to *Vd* (Acciarri et al. 2004; Liu et al. 2015) or complete resistance to *Fom* (Rizza et al. 2002; Rotino et al. 2005).

In eggplant, a few studies aimed at identifying QTL/genes affecting resistance to fungal wilts as well as elucidating the defense responses and signaling pathways activated upon infection have been conducted. Toppino et al. (2008) demonstrated that *Fom* resistance trait introgressed into eggplant from *S. aethiopicum* and *S. integrifolium* was controlled by a single dominant locus (named *Rfo-sa1*), which was mapped on CH02 by Barchi et al. (2010, 2012).

Furthermore, a number of candidate genes involved in early defence responses or signalling pathways activated upon infection have been recently identified in *Fom*-resistant ILs carrying the *Rfo-sal* locus (Barbierato et al. 2016). Boyaci et al. (2010 and 2011) performed a phenotypic characterization and genetic analysis of eggplant lines subjected to *Fom* inoculation. Recently, Miyatake et al. (2016) mapped two *Fusarium* semi-dominant inherited resistance loci (Fukuoka et al. 2012; Hirakawa et al. 2014) on chromosomes E02 and E04 in linkage maps developed from Asian *Fom*-resistant lines (Monma et al. 1996), and the locus on E02 was reported to be orthologous to the *Rfo-sal* locus already mapped on E02. Few studies are available on *Verticillium* resistance. **A first attempt of mapping the resistance to *Verticillium dahliae* was carried out using an interspecific cross between eggplant and the highly tolerant *S. sodomense* (= *S. linneanum*) by developing a first RAPD-AFLP map (Sunseri et al, 2003).** The *Ve* homologous gene of the potato *StVe* was isolated from the wild eggplant species *S. torvum* (Fei et al. 2004), and following deep sequencing of small RNAs, some miRNAs acting in response to *Vd* infection were spotted (Yang et al. 2013). A most recent *de novo* sequencing and transcriptome analysis of roots from *Solanum aculeatissimum* in response to *Vd* (Zhou et al. 2016) revealed the presence of a great number of differentially expressed genes participating in 128 metabolite pathways, among which those related to plant-pathogen interactions, plant-hormone signal transduction and phenylpropanoid biosynthesis are the most represented.

Inter-specific eggplant linkage maps have been constructed and used for the identification of QTL affecting agronomical and fruit quality traits (Doganlar et al. 2002a,b; Frary et al. 2003 and 2014, Wu et al. 2009, Gramazio et al. 2014), however studies aimed at mapping QTL affecting fungal resistance traits to date have been very limited. The first eggplant intra-specific genetic map published by Nunome et al. (2001), and afterwards integrated with various sets of molecular markers (Nunome et al. 2003; Nunome et al. 2009; Fukuoka et al. 2012), was used for mapping two QTL underpinning parthenocarpy and *Fusarium* resistance (Miyatake et al. 2012 and 2016). We also developed a densely populated RAD-tag derived markers map (Barchi et al. 2011) based on an F<sub>2</sub> intra-specific population, which made it possible to identify QTL affecting anthocyanin content (Barchi et al. 2012), key agronomic traits (Portis et al. 2014) as well as biochemical and morphological fruit properties (Toppino et al. 2016). Furthermore, through a GWAs approach, we validated marker/trait associations previously detected and new ones were identified (Cericola et al. 2014, Portis et al. 2015).

The present work aims to locate QTL affecting resistance to *Fusarium oxysporum* f. sp. *melongenae* and *Verticillium dahliae* Kleb in the previously developed intraspecific map, as the female parent (305E40) of the F<sub>2</sub> mapping population bears the *Rfo-sal* locus conferring complete resistance to *Fom* and displays also a partial resistance to *Vd*. On the other hand the male parent ('67/3'), although lacks the *Rfo-sal* locus, revealed a not previously characterized partial

resistance to *Fom* while is highly susceptible to *Verticillium*. The tomato orthologous genes falling within the confidence interval (C.I.) of the discovered QTL were also identified.

## Material and Methods

### Plant material

The starting material was a population of 156 F<sub>2</sub> plants, obtained by selfing the F<sub>1</sub> hybrid derived from the cross between the two eggplant breeding lines '305E40' and '67/3', contrasting for a wide number of key agronomic and metabolic traits (Barchi et al. 2012; Portis et al. 2014; Toppino et al. 2016). The line 305E40 (female parent) is a double haploid derived from an interspecific somatic hybrid *Solanum aethiopicum* gr. gilo(+) *S. melongena* cv. Dourga (Rizza et al. 2002), which was repeatedly backcrossed with the recurrent lines DR2 and Tal1/1, prior to selfing and anther culture. This line carries the locus *Rfo-sal* from *S. aethiopicum*, which confers complete resistance to the soil-borne fungus *Fusarium oxysporum* f. sp. *melongenae* (*Fom*) (Toppino et al. 2008). Moreover, on the basis of our preliminary evaluations, assessed in resistance tests in which the 305E40 was compared to the male parental line 67/3 as well as to eggplant lines which are routinely used in our lab as reference, as they show major or minor manifestations of symptoms (supplemental Figure 1), the line 305E40 shows a partial resistance to *Verticillium dahliae* (*Vd*). Otherwise, the line 67/3 is an F<sub>8</sub> selection from the intra-specific cross between cv. 'Purpura' x cv. 'CIN2', which lacks the *Rfo-sal* locus and is fully susceptible to *Vd*.

In order to assess *Fom* and *Vd* resistance of the F<sub>2</sub> progeny, each of the 156 individuals was selfed and as many F<sub>2:3</sub> progenies obtained. Forty-eight plantlets of each F<sub>2:3</sub> progeny as well as of each parental line and the F<sub>1</sub> hybrid, for a total of 7,632 plantlets, were grown in greenhouse to assess the resistance to *Fom*. Side by side, forty plantlets of each F<sub>2:3</sub> progeny plus the parental lines and the F<sub>1</sub> hybrid, for a total of 6,360 plantlets, were grown in growth chambers to assess the resistance to *Vd*.

### Assessment of *Fusarium oxysporum* f.s. *melongenae* resistance

*Fom* inoculation of the F<sub>2:3</sub> progenies as well as parental lines and the F<sub>1</sub> hybrid progenies was performed at Montanaso Lombardo (45°20'12"N 9°28'11"E, Italy), according to the dip-root method reported by Cappelli et al. (1995). Plantlets at 2-3th true leaf stage, grown in pasteurized peat in 104 holes plastic trays, were gently removed, their roots washed under running tap water and, after inoculation with *Fom* isolate (the same used by Cappelli et al. 1995) at concentration of 1.5x10<sup>6</sup> conidia/ml for 15 minutes, were transferred in 54 holes plastic trays filled with pasteurized peat (1:1, v/v). Plantlets were arranged in two randomized complete blocks in greenhouse, with 24 plants for each F<sub>2:3</sub> progeny per

block. At 30 Days After Inoculation (DAI), each plantlet was assessed and its degree of *Fom* infection was scored according to a scale ranging from 1 to 0, where 1 is correspond to “fully resistant plant with complete absence of symptoms”, 0 to “dead plant” with the intermediate values as follows: 0.9 = some spot of yellowing in basal leaves, absence of symptoms in intermediate and upper ones; 0.8 = extended yellowing in basal leaves; 0.7 = extended yellowing in basal leaves and some spot of yellowing in intermediate ones; 0.6 = extended yellowing in both basal and intermediate leaves; 0.5= some spot of necrosis in basal leaves, extended yellowing in basal and intermediate leaves and some spot of yellowing in upper ones; 0.4 = partial necrosis in basal leaves, extended yellowing in intermediate and upper ones; 0.3 = necrosis in basal leaves and some spot of necrosis in intermediate ones; 0.2 = necrosis in basal and intermediate leaves, falling of basal leaves; 0.1 = complete necrosis in all the leaves, falling of basal and intermediate leaves.

For each block, the resistance ratio was calculated as follows:

$$R = \frac{\sum (\text{plant} * \text{score assigned})}{\text{total n}^\circ \text{ of inoculated plants}} * 100$$

#### Assessment of *Verticillium dahliae* resistance

*Vd* inoculation of the F<sub>2,3</sub> progenies as well as parental lines and the F<sub>1</sub> hybrid was performed according to a root-dip method at Carmagnola (44°53'N; 7°41'E, Italy). Plantlets at 2-3th true leaf stage, grown as previously described, were inoculated with *Verticillium dahliae* Kleb isolate (eggplant isolate V7) by dipping roots in a conidial suspension at a concentration of 5x10<sup>5</sup> conidia/ml for 15 minutes, and transferred in 15 cm-diam plastic pots filled with a pasteurized mixture of sand and soil (1:1, v/v) containing NPK fertilizer granules. Plantlets were then arranged in two randomized complete blocks in two separates growth chambers, with 20 plants per each entry (F<sub>2</sub> individual) per block and kept at 25°C ± 2°C day, 20°C ± 2°C night, 50 μEm-2 s<sup>-1</sup> with a 12-h photoperiod. The severity of *Vd* symptoms was evaluated on each leaf of each plant after 20 (early response) and 40 (late response) DAI. The symptomatic leaves were classified in a scale ranging from 0 to 5 as follows: 0 = no necrosis or chlorosis; 1= asymmetry and/or chlorosis on ¼ of a leaf, 2 = chlorosis on ½ of a leaf, 3= chlorosis on more than ½ of a leaf, 4 = wilted or partially necrotic leaf, 5 = necrotic or dead leaf.

#### Statistical analyses, molecular mapping and QTL detection

Statistical analyses were performed using R software (Team 2009). A conventional analysis of variance was applied to estimate genotype and environment effects based on the linear model  $Y_{ij} = \mu + g_i + b_j + e_{ij}$ , where  $\mu$ ,  $g$ ,  $b$  and  $e$

represent, respectively, the overall mean, the genotypic effect, the block effect and the error. Broad-sense heritability values were given by  $\sigma^2_G/[\sigma^2_G + (\sigma^2_E/n)]$ , where  $\sigma^2_G$  represents the genetic variance,  $\sigma^2_E$  the residual variance and  $n$  the number of blocks. Correlations between traits were estimated using the Spearman coefficient, and normality, kurtosis and skewness were assessed with the Shapiro-Wilks test ( $\alpha=0.05$ ). Segregation was considered as transgressive when at least the scoring of one  $F_2$  individual was higher or lower by at least two standard deviations than the higher or lower scoring of a parental line.

Sequence comparison between orthologs of the tomato candidate genes falling within the C.I. of the *FMI* QTL as reported in Miyatake et al. (2015) yielded polymorphisms between 305E40 and 67/3, which allowed to develop three new HRM markers from the tomato genes Solyc02g032030.1, Solyc02g032200.2 and Solyc02g037540.1 (primers sequence in Supplementary Table 1). These three markers were added to our previously developed genetic map (Barchi et al. 2012), using the software JoinMap v4.0 (van Ooijen J. 2006) and mapped on E02 (see Figure 1). This newly developed map, which includes 418 makers (339 SNPs, 5 HRMs, 3 CAPSs, 11 RFLPs, 33 SSRs and 27 COSII) and spans 1,390 cM., was the basis for QTL analyses.

QTL analyses for *Fom* resistance was based on the values of the resistance ratios while *Vd* resistance data were treated as adjusted line means (best linear unbiased predictors). Several multivariate linear mixed models were tested using a combination of the F-test (for the fixed component) and the Akaike test (for the random component). For both *Fom* and *Vd* resistance the best fit model was:  $pib = rb + gi + e$ , where *pib* represents the phenotype of the  $b^{\text{th}}$  replicate of the  $i^{\text{th}}$  genotype; *rb* the fixed effect of the  $b^{\text{th}}$  replicate; *gi* the random effect of the  $i^{\text{th}}$  genotype, and *e* the residual.

Putative QTL location was determined by both interval (Lander and Botstein 1989) and MQM (Jansen 1993; Jansen and Stam 1994) mapping, as implemented in MapQTL v5 software (Van Ooijen 2004). QTL were initially identified using interval mapping, afterwards one linked marker per putative QTL was treated as a co-factor in the approximate multiple QTL model. Co-factor selection and MQM analysis were repeated until no new QTL could be identified. LOD thresholds for declaring a QTL to be significant at the 5% genome-wide probability level were established empirically by applying 1,000 permutations per trait (Churchill and Doerge 1994). Additive and dominance genetic effects, as well as the percentage of the phenotypic variance (PV) explained by each QTL, were obtained from the final multiple QTL model. Individual QTL were prefixed by a trait abbreviation (*Fom* or *Vd*), followed by the chromosome designation and, according to the scoring for *Vd* inoculation, were suffixed as “20 DAI” or “40 DAI”. Circos reporting the resulting map based on the Krzywinski et al. (2009) software was drawn. Syntenic regions of the tomato genome (sequence build 2.50; [http://solgenomics.net/organism/Solanum\\_lycopersicum/genome](http://solgenomics.net/organism/Solanum_lycopersicum/genome)) were accessed by blastN to identify candidate genes co-localizing within the Interval Mapping (IM) of eggplant QTL. Initial searches were conducted



using 20 kb intervals, then narrowed to 10 kb for intervals of interest. Putative tomato orthologous of the eggplant genes were identified by Blast search in the tomato gene indices at DFCI (<http://compbio.dfci.harvard.edu/tgi/>).

## Results

Phenotypic variation and inter-trait correlations.

Trait codes, their correspondent values, broad sense heritability and the number of transgressive individuals in respect to the two parental lines are shown in Table 1. As expected, the line '305E40' which carries the locus *Rfo-Sa1*, displayed a complete resistance against *Fom* but also a partial resistance against *Vd*, as at both 20 and 40 days after *Vd* inoculation, plantlets showed low or moderate symptoms of infection. Unexpectedly, the "67/3" plantlets survived to *Fom* inoculation and, although showed a reduced growth and yellowish leaves, displayed a partial resistance which was not described before; on the other hand they exhibited a high sensitivity to *Vd* at both 20 and 40 DAI.

The F<sub>1</sub> plantlets revealed a resistance analogous to the female parent '305E40' in respect to *Fom*, while intermediate between the parental lines in respect to *Vd*. Based on the assessment of the F<sub>2:3</sub> progenies, some F<sub>2</sub> individuals resulted highly sensitive to *Vd* inoculation and transgressive even in respect to the most sensitive parent 67/3, both at 20 and 40 DAI (10 and 8 plants respectively), since their progenies were unable to survive after 30 DAI. As regard resistance to *Fom*, transgressive segregation in respect to the most sensitive parent 67/3 was detected in 51 F<sub>2</sub> individuals.

The broad sense heritability values were high for all the three traits in study, ranging from 0.985 (*Vd40*) to 1 (*Fom*) (Table 1). Significant positive inter-trait correlations ( $p < 0.05$ ) were detected for *Vd20/Vd40* (0.287) and *Vd20/Fom* (0.25) (Table 2).

QTL affecting *Fusarium* resistance in eggplant.

Two QTL related to *Fom* resistance were mapped in the F<sub>2</sub> population (Table 3 and Fig. 1). A major QTL (*FomE02.01*), explaining ~68% of the PV and derived from the female parental line '305E40' lies on E2. A further major QTL (*FomE011.01*), which explains about 11% of the PV, but derives from the male parental line '67/3', was located on E11.

QTL affecting *Verticillium* resistance in eggplant.

A major QTL involved in resistance to *Vd* at 20 DAI (*Vd20E08.01*) was mapped on E08 (top segment), which

explains ~11% of the PV. At 40 DAI, a major QTL, (*Vd40E05.01*), explaining 20.7 % of PV, was located on E05. Furthermore, a minor QTL (*Vd40E09.01*) explaining 7.3 of the PV mapped on E09. All of the identified QTL derived from the female parental line ‘305E40’ (Table 3 and Fig. 1).

Candidate genes identification based on orthology with tomato.

The tomato (build 2.50; [http://solgenomics.net/organism/Solanum\\_lycopersicum/genome](http://solgenomics.net/organism/Solanum_lycopersicum/genome)) orthologous sequences, syntenic to the eggplant regions underlying the identified QTL, were mined for NLR (also known as NB-LRR or NBS-LRR) and/or any other conserved domain associated to resistance (R) genes, as well by entering the keyword “resistance”, in order to identify candidate genes putatively involved in the control of the traits in study. The identified tomato candidate genes are reported in Table 4.

The QTL *FomE02.1* was found to be bounded by markers Solyc02g037540.1 and Solyc02g032200.2, which are located at 0.3cM from each other. This interval is orthologous to a region of 3Mbp on T2, with the former marker mapping at 31,080,754 and the latter at 28,002,925. Within this interval  $\pm 0.5$ Mb (27.5Mb to 31.5Mb), 10 genes associated to resistance in tomato were identified. The QTL *FomE011.1* was linked to the marker C2\_At3g51010, which maps to its orthologous T4 region at 2,426,283 Mb. In a neighbour-hood of 1Mb, 21 tomato orthologous genes associated to resistance were spotted.

The QTL *Vd20E08.1* was found associated to the marker 18202\_PstI\_L304, which maps at 1,902,945 on T8. At 1Mb around these coordinates, 5 genes associated to NLR or to other annotated ‘R domain’ were identified. The QTL *Vd40E05.1* was bounded by two markers: 10016\_PstI\_L402 and 12391\_PstI\_L355, mapping on T12 at 66,653,563 and 66,128,270 respectively. Ten genes annotated as associated to resistance were detected in a 1Mb around these coordinates. The QTL *Vd40E09.1* was associated to the marker 32063\_PstI\_L393, which maps at 7,0144,642 on T9 and in the 1Mb around this coordinate, three genes associated to resistance were spotted.

## Discussion

The two fungi *Fusarium oxysporum* f. sp. *melongenae* (*Fom*) and *Verticillium dahliae* (*Vd*) are among the most serious and widespread diseases in eggplant, also due to the fact can persist in the soil for many years. Currently, there is no robust single control measure by which to manage both pathogens, even because diverse isolates express different levels of virulence (Michielse & Rep 2009; Altinok et al. 2010, 2013, 2014). Crop rotation, fumigation and fungicide applications can reduce the risk of infection, however they are not highly effective, consistent and are environmentally impactful (Fradin et al. 2009; King et al. 2010). A possible alternative is represented by grafting cultivated eggplant

varieties on resistant rootstocks, which has become a common practice mostly in greenhouse cultivation. However, this practice is time consuming, expensive and may influence plant vigor, yield and product quality (Villeneuve et al., 2014). An ideal alternative is represented by the development of genetically resistant cultivars; thus, the identification of the QTL affecting resistance and candidate genes playing a key role in the plant response to the infection, combined with the development of molecular markers strictly linked to the resistance trait, can substantially speed up breeding programs aimed at the obtainment of improved resistant varieties.

*Verticillium* and *Fusarium* resistance traits have been extensively studied in the *Solanaceous* crop tomato. With regard to *Verticillium*, the resistance genes *Ve1* and *Ve2* were cloned, but only *Ve1* was found to provide resistance against race 1 strains of *V. dahliae* and *V. albo-atrum* (Diwan et al. 1999; Kawchuk et al. 2001; Fradin et al. 2009). The sequence information of the two genes was also used to amplify candidate *Ve* orthologs in potato, and markers to track resistance in potato germplasm developed (Bae et al. 2008; Uribe et al. 2014). *Ve1* encodes an extracellular leucine-rich repeat (eLRR) receptor-like protein (RLP), that serves as a cell surface receptor for recognition of the secreted *Verticillium* effector Ave1 (Fradin, 2009). Several other *Ve*-similar genes from different species have been identified in the last years (Bae et al. 2008; Vining and Davis 2009; Hayes et al. 2011; Zhang et al. 2012), suggesting a common role of these genes in resistance against *Verticillium* wilt. QTL and four resistance genes against *Fom* were identified in the tomato relatives *Solanum pennellii* and *Solanum pimpinellifolium* (Sarfatti et al. 1989 and 1991; Bournival et al. 1990; Segal et al. 1992; Ori et al. 1997; Hemming et al. 2004; Lim et al. 2008) and the *I2* gene, a member of the tomato I2C multigenic cluster coding for a NBS-LRR protein, was found to confer full resistance to *Fusarium oxysporum f.sp. lycopersici* race 2 (Simons et al. 1998).

At present no effective resistance gene(s) against *Verticillium* wilt have been detected in the *S. melongena* germplasm, although some allied species, such as *S. linnaeanum*, *S. aculeatissimum*, *S. sisymbriifolium* and *S. torvum*, exhibit different levels of resistance to the pathogen (Daunay 2008). The latter may thus represent a key source for the introgression of the resistance traits into cultivated varieties. *Verticillium* resistance was transferred from the wild species *S. linneanum* (syn. *S. sodomaeum*) to cultivated eggplants; advanced breeding lines carrying partial resistance to *Vd* from this wild species were also established (Acciarri et al. 2001 and 2004), and a gene specific marker for the *Ve* homolog developed (Liu et al. 2015).

We identified three new QTL involved in the early and late response to *Vd* inoculation, namely *Vd20E08*, *Vd40E05.1* and *Vd40E09*, which are located on E8, E5 and E9 respectively. These results demonstrate that presumably different genomic regions are involved in the interaction between *Vd* and *S. melongena* during the time-course of their interaction offering the possibility to better steer both the study and the breeding activity to improve the resistance to

*Verticillium*. The QTL analysis revealed larger CI for these QTL when compared to the ones we detected to be involved in resistance to *Fom*, presumably because of a minor phenotypic variation detected between the parents of our mapping population in respect to the one observed for *Fom*. Notwithstanding, synteny analyses with tomato revealed that the regions corresponding to all the eggplant QTL for resistance to *Vd* contain clusters of genes associated to “resistance” or “LRR”, suggesting that also these regions may play an important role in the defence mechanism in tomato, and presumably also in eggplant. However the identified eggplant *Vd40E09* QTL, which maps at ~7.7Mb on the tomato chromosome T9, plays only a small role in resistance to *Vd* and from our synteny analysis it does not correspond to the region containing the tomato *Ve1* (reported as major resistance gene) and *Ve2* genes, which are located on the top of chromosome T9.

Although the resistant parent of our mapping population did not display a marked resistance against *Vd*, we identified a major resistance QTL on chromosome E5, which explains about 20% of the PV. The latter represents the first major QTL involved in resistance to *Verticillium* spp. in eggplant and, although deserving a more detailed characterization, it can be considered a starting point for dissecting the resistance trait against the pathogen.

The first sources of resistance to *Fom* in eggplant were identified in wild and allied species (Daunay et al. 1991). An example is represented by the resistance trait to *Fom* introgressed into cultivated eggplant from *S. aethiopicum* and *S. integrifolium* through somatic hybridization followed by anther culture of the tetraploid somatic hybrids for obtaining dihaploid plants (Rizza et al. 2002). Advanced introgression lines were then developed through backcrosses with recurrent *S. melongena* lines, followed by selfing and/or anther culture to obtain pure lines (Rotino et al., 2014). Genes conferring partial resistance to *Fom* were also detected in Asian landraces (Komochi et al. 1996; Monma et al. 1996 and 1997), which were associated with genetic markers and introgressed in European eggplant genotypes (Mutlu et al. 2008). One of these lines (LS1934) was employed to develop “Daitaro” and “Daizaburou”, two resistant eggplant rootstocks (Yoshida et al. 2004).

The female parent (line ‘305E40’) of our mapping population is an introgression line carrying the resistance locus *Rfo-sa1* derived from *Solanum aethiopicum* and is fully resistant to *Fom*, as it grows vigorously and symptomless after *Fusarium* inoculation. On the other side, the male parental line ‘67/3’, which was thought to be fully sensitive to the pathogen, unexpectedly displayed a partial resistance to *Fom*, as when inoculated showed evident symptoms and reduced growth, but survived at 30 DAI and beyond.

We detected a QTL on chromosome E2, namely *FomE02.1*, which explains about 70% of the phenotypic variation and derived from the resistant parent “305E40”. This QTL is located at just 1.2cM away from the locus *Rfo-sal* and co-maps with resistance locus *FMI* at the end of chromosome 2, (Miyatake et al. 2015).

Due to the availability of the eggplant genome sequence (Hirakawa et al., 2014), Miyatake et al. (2016) explored the synteny between eggplant and tomato and identified 25 tomato resistance genes syntenic with the eggplant genomic region of *FMI*. The latter spans a tomato genomic region of about 29Mb, suggesting that additional knowledge on eggplant genome is required, also in view of exploiting the resistance genes present in allied species such as *S. aethiopicum* (Gramazio et al. 2016), *S. torvum* (Yang et al. 2014) and *S. aculeatissimum* (Zhou et al. 2016). By comparing the eggplant homologous sequences of these tomato genes in our parental lines, we developed three new molecular markers which were added to our genetic map (Barchi et al., 2012). In this new map, the *FusE02.1* QTL shows a much smaller CI in respect to *FMI*, and corresponds to a tomato syntenic region of approximately 1.8Mb, which is 16 fold narrower than the one previously identified by Miyatake et al (2016) and contains 10 genes annotated as ‘NLR’ or ‘resistance’ or both.

The activation of defense mechanisms requires pathogen detection using either cell surface or intracellular receptors. Most disease resistance (R) genes encode for proteins belonging to the nucleotide-binding, leucine-rich repeat protein families, which directly or indirectly recognize pathogen effectors and activate a range of defence responses through different signalling domains at their N termini (TIR-NLRs or CC-NLRs). It is well known that plant genomes contain hundreds of NLR-encoding genes and genes encoding for proteins having a role in diverse signalling pathways, leading to plant defence responses. Indeed, clusters of genes involved in redox and lipid metabolism as well as transcription factors were identified in the *FusE02.1* region (data not shown). This result is in accordance with the recent finding that, following *F. oxysporum* infection, a basal molecular response to pathogens occurs, involving recognition of the pathogen in the cell surface and/or the modulation of genes related to both redox state maintenance and cell wall modification and composition (Barbierato et al., 2016). Presumably, the gene responsible for resistance mediates a rapid response at the site of infection and activates a defensive pathway, which in turn protects the plant from further diffusion of the pathogen (Goggin et al. 2006). This hypothesis is supported by the evidence that the *Rfo-Sal* mediated response activated upon inoculation with *Fusarium* was able to improve the reaction of eggplant plantlets also against *Verticillium* attacks (Barbierato et al. 2016).

The resistance trait associated to the QTL *FomE02.1* segregated as a single Mendelian dominant trait in our F<sub>2</sub> mapping population ( $\chi^2 = 1.44$  P=0.05) as well as in crosses between introgression lines holding the resistance locus and

fully susceptible eggplant genotypes (Toppino et al. 2008; data not shown), and the same does the locus FM1. This seems to confirm that in both *RfoSal1* and *FM1* loci only one gene is responsible for the resistance trait against *Fusarium*. However, it must be stressed that R genes commonly reside in complex clusters making it difficult the dissection of clustered genes conferring resistance (Kawashima et al. 2016). Moreover, as previously reported (Portis et al; 2014), the line ‘305E40’ carries, in the region hosting the *FomE02.1* QTL, an introgressed portion of the *S. aethiopicum* genome, which might represent an additional source of resistance genes compared to the orthologous eggplant region.

We also detected on E11 a new major QTL, namely *FomE11.1*, which is involved in the *Fusarium* resistance and explains about 11% of the PV. This resistance trait derives from the male parental line 67/3, which showed partial resistance following *Fusarium* inoculation. The partial resistance was also detected in several F3 progenies derived from F2 plants that were heterozygous or lacked the *Rfo-sal1* locus (see supplemental Table 2). The response mediated by this trait seems to act differently, by avoiding the lethal outcome of the infection but with an evident manifestation of symptoms. A total of 21 genes annotated as ‘NLR’ or ‘resistance’ or both were identified in the *FomE11.1* CI; however, none of them corresponds to the tomato *I2* gene, which is located at the bottom of T11. Unfortunately we were not able to study the pattern of inheritance of this locus, as its effect was masked in the F1 and the employed population by the dominant allele of the major QTL *FusE02.1* derived from ‘305E40’, thus further studies are needed to better elucidate its genetic bases in an *ad hoc* developed segregant population. The availability of multiple functional polymorphisms associated to a single resistance gene as well as the presence of independent genes conferring resistance to *Fom* is of great interest for breeding (Fukuoka et al. 2014), thus the deep mining of allelic variation is of substantial importance for the development of superior lines carrying a durable resistance conferred by pyramided traits of resistance (Fukuoka et al. 2015).

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Table 1. List of the traits analysed and their code, means, standard deviations (SD), coefficients of variation (cv), broad sense heritability and number of transgressive individuals respect to parents. Significant mean difference among parental values according to Wilcoxon test (\*p<0.05) is reported. Skewness, kurtosis (with their standard errors (SE)) and Shapiro-Wilks test are also listed.

Trait	Trait code	Parents mean $\pm$ SD		Significance Wilcoxon test	F1	F2 population mean $\pm$ SD	cv	Shapiro Wilks	Skewness	SE	Kurtosis	SE	Heritability	Transgressive respect 305E40	Transgressive respect 67/3
		305E40	67/3												
<i>Fusarium</i>	<i>Fom</i>	1 $\pm$ 0	0.6 $\pm$ 0	yes	1 $\pm$ 0	0.68 $\pm$ 0.30	0.46	<0.01	-0.87	0.190	-0.25	0.39	1	-	51
<i>Verticillium</i> 20 DAI	<i>Vd20</i>	1.93 $\pm$ 0.99	3.14 $\pm$ 0.81	yes	2.73 $\pm$ 1.10	2.77 $\pm$ 0.62	0.22	<0.01	-0.70	0.198	0.88	0.39	0.87	-	10
<i>Verticillium</i> 40 DAI	<i>Vd40</i>	2.3 $\pm$ 0.96	3.69 $\pm$ 0.97	yes	2.75 $\pm$ 1.23	2.98 $\pm$ 0.81	0.27	<0.01	0.47	0.198	-0.09	0.39	0.85	-	8



Table 2. Inter-trait Spearman correlations assessed in the mapping population. Correlations are significant (\*at  $p < 0.05$  and \*\* at  $p > 0.01$ ).

	<b>Vd40</b>	<b>Fus</b>
<b>Vd20</b>	0.287**	0.250**
<b>Vd40</b>		0.094

Table 3. QTL detected in the mapping population. For each trait the chromosomal location (CH), the genome-wide thresholds (GW) at  $p=0.05$  (as determined from 1,000 permutations) are indicated. The closest mapping marker to each QTL are indicated, along with the confidence interval (CI), the LOD, the percentage of phenotypic variation explained (PV) and the additive (A)/dominance (D) contribution.

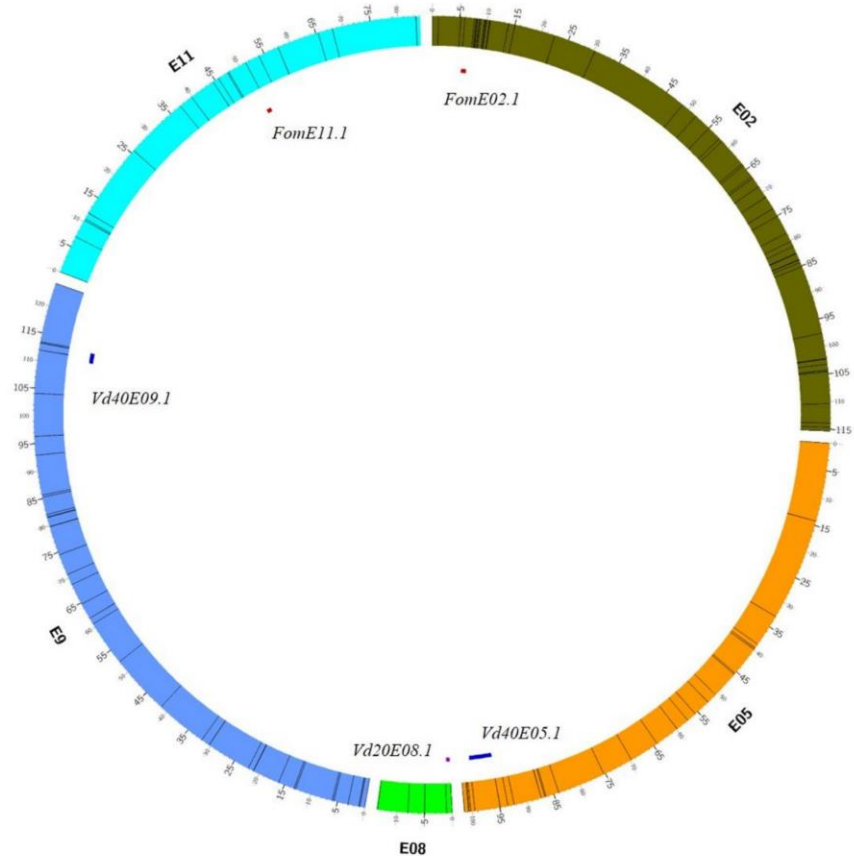
Trait	CH	GW	QTL	Position	Locus	CI	LOD	PV	A	D
Fusarium	2	3.9	<i>FomE02.01</i>	6.595	Solyc02G032030.1	6.59-6.85	53.38	67,6	33.379	10.588
Fusarium	11		<i>FomE11.01</i>	51.313	C2_At3g51010	50.8-51.3	16.04	11.1	-13.312	5.101
Verticillium 20d	8	3.1	<i>Vd20E08.1</i>	0	18202_PstI_L304	0-1.0	3.70	10.8	0.04214	0.035
Verticillium 40d	5	3.1	<i>Vd40E05.1</i>	99.515	10016_PstI_L402	94.9-99.5	7.93	20.7	0.054	0.008
Verticillium 40d	9	3.1	<i>Ver40E09.1</i>	111.872	32063_PstI_L393	106-112.8	3.14	7.3	0.0021	0.045

Table 4. Candidate tomato ‘resistance’ genes according to Solgenomic network database for the 5 identified QTL in eggplant on the basis of their mapping positions.

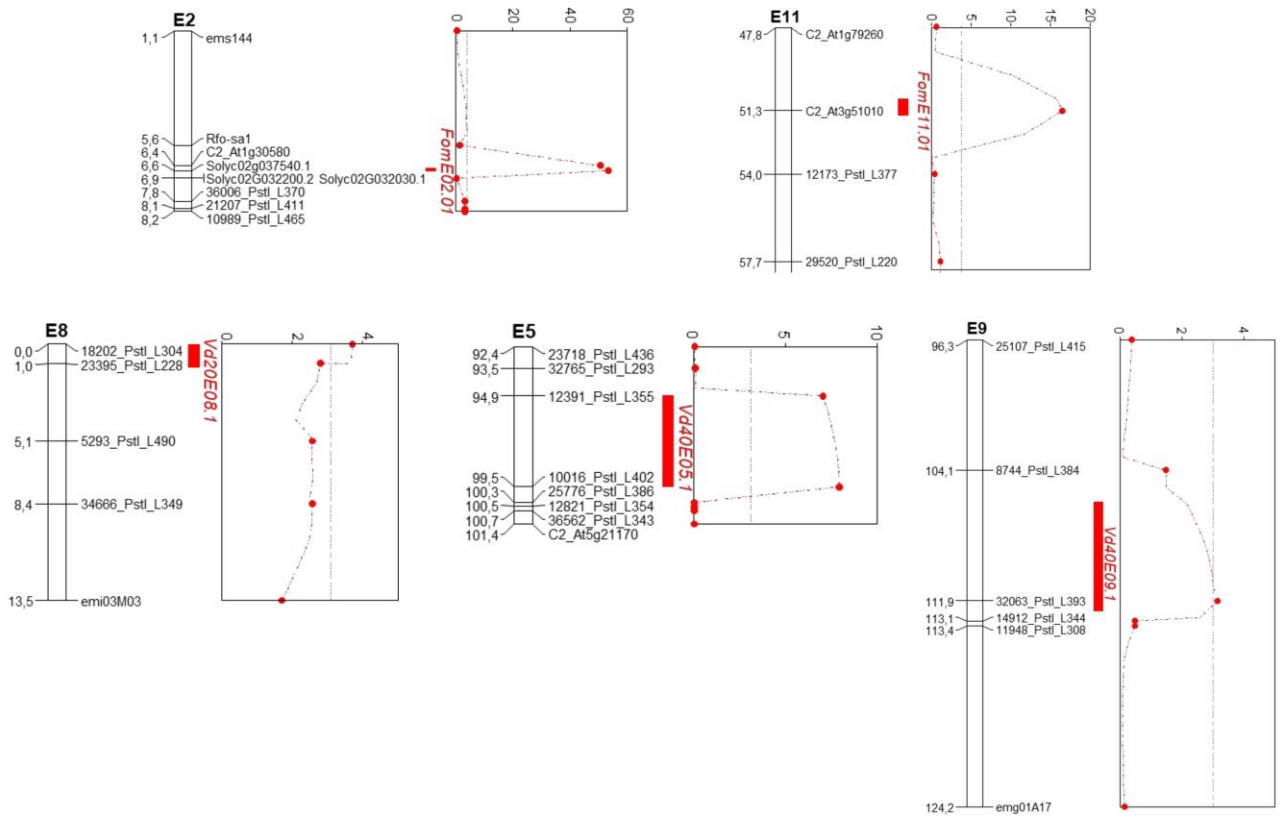
<b>QTL</b>	<b>Tomato genes</b>	<b>Start on SL2.5</b>	<b>End on SL2.5</b>	<b>Annotation</b>
<b><i>FomE02.1</i></b>	Solyc02g032230.1.1	28020596	28021489	Lrr resistance protein fragment
	Solyc02g032240.1.1	28040508	28041894	Nbs resistance protein fragment
	Solyc02g032250.1.1	28051885	28052101	Tir resistance protein fragment
	Solyc02g032260.1.1	28059099	28059426	Nbs resistance protein fragment
	Solyc02g032640.1.1	28903072	28903603	Tir resistance protein fragment
	Solyc02g032650.2.1	28907110	28910721	Nbs-lrr resistance protein
	Solyc02g036270.2.1	30478478	30481057	Cc-nbs-lrr resistance protein
	Solyc02g036280.2.1	30480566	30483338	Lrr resistance protein fragment
	Solyc02g037540.1.1	31080753	31083828	Cc-nbs-lrr resistance protein
<b><i>FomE11.1</i></b>	Solyc04g008830.1.1	2438052	2439834	LRR receptor-like serine/threonine-protein kinase RLP
	Solyc04g008980.2.1	2567072	2569411	F-box/LRR-repeat protein (AHRD V1 ***- C0S347_PARBP
	Solyc04g009070.1.1	2629301	2629574	Nbs resistance protein fragment
	Solyc04g009080.1.1	2629627	2629996	Nbs resistance protein fragment
	Solyc04g009090.1.1	2630934	2632218	Nbs-lrr resistance protein
	Solyc04g009100.1.1	2632840	2633663	Nbs resistance protein fragment
	Solyc04g009110.1.1	2637078	2639610	Cc-nbs-lrr resistance protein
	Solyc04g009120.1.1	2641912	2644352	Cc-nbs-lrr resistance protein
	Solyc04g009130.2.1	2646822	2649379	Cc-nbs-lrr resistance protein
	Solyc04g009150.1.1	2655517	2658052	Cc-nbs-lrr resistance protein
	Solyc04g009240.1.1	2713464	2716002	Cc-nbs-lrr resistance protein
	Solyc04g009250.1.1	2719884	2722407	Cc-nbs-lrr resistance protein
	Solyc04g009260.1.1	2728029	2730579	Cc-nbs-lrr resistance protein
	Solyc04g009270.2.1	2733651	2734643	Cc-nbs resistance protein fragment
	Solyc04g009290.1.1	2735949	2738484	Cc-nbs-lrr resistance protein
	Solyc11g006520.1.1	1181635	1184459	Cc-nbs-lrr, resistance protein
	Solyc11g006530.1.1	1186570	1189847	Cc-nbs-lrr, resistance protein
	Solyc11g006630.1.1	1237303	1240179	Cc-nbs-lrr, resistance protein

	Solyc11g006640.1.1	1242255	1245275	Cc-nbs-lrr, resistance protein
	Solyc11g007140.1.1	1589660	1593190	Tetracycline resistance protein (AHRD V1 ***- A4EIK2_9RHOB)
	Solyc11g007790.1.1	2028382	2031076	Nbs-lrr, resistance protein
<b>Vd20E08.1</b>	Solyc08g006920.1.1	1492120	1494699	F-box/LRR-repeat protein 4 (AHRD V1 ***- FBL4_ARATH); contains Interpro domain(s) IPR013101 Leucine-rich repeat 2
	Solyc08g006970.2.1	1548921	1552302	Lrr resistance protein fragment
	Solyc08g007250.1.1	1830375	1835999	Cc-nbs-lrr resistance protein
	Solyc08g007630.1.1	2161134	2164101	Cc-nbs-lrr resistance protein
	Solyc08g007640.1.1	2164225	2165170	Lrr resistance protein fragment
<b>Vd40E05.1</b>	Solyc12g099040.1.1	66343088	66345132	Lrr, resistance protein fragment
	Solyc12g099060.1.1	66349196	66350379	NBS-type resistance protein RGC2 (AHRD V1 ***- C3RVU3_MUSAC)
	Solyc12g099480.1.1	66616899	66620554	Bifunctional polymyxin resistance protein ArnA (AHRD V1 ***- ARNA_PHOLL); contains Interpro domain(s) IPR016040 NAD(P)-binding domain
	Solyc12g099870.1.1	66833208	66835311	LRR receptor-like serine/threonine-protein kinase, RLP
	Solyc12g099880.1.1	66836304	66836628	Lrr, resistance protein fragment
	Solyc12g099950.1.1	66893905	66896670	LRR receptor-like serine/threonine-protein kinase, RLP
	Solyc12g099980.1.1	66904985	66907921	LRR receptor-like serine/threonine-protein kinase, RLP
	Solyc12g100010.1.1	66918120	66922325	LRR receptor-like serine/threonine-protein kinase, RLP
	Solyc12g100020.1.1	66926078	66928650	LRR receptor-like serine/threonine-protein kinase, RLP
	Solyc12g100030.1.1	66930506	66933308	LRR receptor-like serine/threonine-protein kinase, RLP
	Solyc09g090620.1.1	70078075	70078675	CC-NBS-LRR class disease resistance protein (AHRD V1 ***- C6FF62_SOYBN)
	Solyc09g090670.2.1	70121288	70125663	Oxidation resistance 1-like protein (AHRD V1 ***- Q6Z157_ORYSJ); contains Interpro domain(s) IPR006571 TLDC
	Solyc09g091210.2.1	70523220	70524706	Disease resistance response/ dirigent-like protein (AHRD V1 ***- Q0WPQ6_ARATH); contains Interpro domain(s) IPR004265 Plant disease resistance response protein
<b>Vd40E09.1</b>	Solyc09g090620.1.1	70078075	70078675	CC-NBS-LRR class disease resistance protein (AHRD V1 ***- C6FF62_SOYBN)
	Solyc09g090670.2.1	70121288	70125663	Oxidation resistance 1-like protein (AHRD V1 ***- Q6Z157_ORYSJ); contains Interpro domain(s) IPR006571 TLDC
	Solyc09g091210.2.1	70523220	70524706	Disease resistance response/ dirigent-like protein (AHRD V1 ***- Q0WPQ6_ARATH); contains Interpro domain(s) IPR004265 Plant disease resistance response protein

A



B



**Fig. 1 A) Eggplant chromosomes containing the *Fom*, *Vd20* and *Vd40* QTL detected. Each line inside the chromosome represents a molecular markers, with map distances (in cM) shown outside each chromosome. The width of the bars represents the confidence interval of the QTL (LODmax<sup>-1</sup> interval). B) Detailed regions of *Fom*, *Vd20* and *Vd40* QTL, with marker information, including the position of the three new HRM markers developed, and genetic distances. The width of the bars represents the confidence interval of the QTL (LODmax<sup>-1</sup> interval), while the graphs on right side of each region represent the plot of LOD values for the regions considered, together with the GW thresholds for declaring a QTL to be significant at the 5% genome-wide probability level.**