**Epistatic determinism of durum wheat resistance to the Wheat Spindle Streak Mosaic Virus**

### *Yan Holtz1\*, Michel Bonnefoy3, Véronique Viader2, Morgane Ardisson2, Nicolas O. Rode*2*, Gérard Poux2, Pierre Roumet2, Véronique Marie-Jeanne2, Vincent Ranwez1, Sylvain Santoni2, David Gouache3, Jacques L. David1\**

1 Montpellier SupAgro, UMR AGAP, F-34060 Montpellier (France)

2 INRA, UMR AGAP, F-34060 Montpellier (France)

*3* Arvalis, institut du Végétal

\* Corresponding author

E-mail:

Yan.holtz@supagro.fr

[Jacques.david@supagro.fr](mailto:Jacques.david@supagro.fr)

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**Abstract**

*Wheat spindle streak mosaic virus* (WSSMV) is a major disease for durum wheat in Europe and North America. Breeding is currently the only way to fight the virus since no treatment is available. This paper studies the genetic determinism of WSSMV resistance using two populations of durum wheat obtained by crossing two elite varieties with a WSSMV resistant emmer cultivar. In 2012 and 2015, 347 Recombinant Inbred Lines (RIL) were phenotyped using visual notations, ELISA and qPCR and genotyped using locus targeted capture. This allows us to build a consensus genetic map of 8568 markers and to identify three chromosomal regions involved in the WSSMV resistance. The two major regions, located on chromosome 7A and 7B, explain together through epistatic interaction up to 43% of the phenotypic variation. Flanking sequences of our genetic markers are provided to ease future marker assisted selection of WSSMV resistant cultivars.

**Introduction**

*Wheat spindle streak mosaic virus* (WSSMV) infects bread and durum wheats in Europe and North America (Ordon et al. 2009). It is a *Bymovirus* in the family of *Potyviridae* (Barnett; Hariri et al. 1996; Xiaoyun et al. 1998) that causes yellow-stripes mosaic patterns on leaves and stunted spring growth, which results in extensive damages and yield losses (ref XXX). Since breeding resistant cultivars appears currently as the only way to fight the disease (Kanyuka et al, 2003.), it is crucial to find resistant progenitors and to understand the genetic determinism of their resistance.

WSSMV is transmitted by *Polymyxa graminis* (Adams 1990)*,* a *Plasmodiophoridae* (Schwelm et al. 2015) that resides in the soil and can shelter the virus for ten years or more (Kanyuka et al. 2003; Driskel et al. 2004). The infection first starts in roots, and then progresses in the aerial part of the plant up to the leaves (Carroll et al. 1997). Mosaic symptoms are mostly visible in leaves and characterized by cytoplasmic inclusions in infected cells (Sohn et al. 1995).

*Polymyxa graminis* is also the vector of the *Wheat yellow mosaic virus* (WYMV), which is described mainly in Asia, and *Barley Yellow Mosaic Virus* (BaYMV). Those two bymovirus share about 70% of their genes with WSSMV (Xiaoyun et al. 1998)(Sohn et al. 1995). A few furovirus also share important genomic similarities with WSSMV as well as a similar life cycle: *Soil-borne cereal mosaic virus* (SBCMV) and *Soil-borne wheat mosaic virus (SBWMV)* (Kanyuka et al. 2003)*.*

In the allohexaploid bread wheat, (*Triticum aestivum*, 2n=6X=42, AABBDD genomes), SBCMV resistance is attributed to two main loci named *Sbm1* and *Sbm2* located on chromosome 5DL and 2BS respectively (Bass et al. 2006; Perovic et al. 2009)*.* The resistance is known to be a translocation resistance: the plant blocks the virus progression along the xylem and thus circumvents its accumulation in the plant roots (Liu et al.).

In the allotetraploid durum wheat, (*Triticum turgidum durum*, 2n=4X=28, AABB genomes), this SBMV resistance is controlled by a major QTL on chromosome 2BS and minor loci) (Maccaferri et al. 2011a; Taylor et al. 2011; Maccaferri et al. 2011b).

Concerning WSSMV, a major QTL was detected in bread wheat on chromosome 2DL for resistance to both WSSMV (Khan et al. 2000) and WYMV (Kojima et al. 2015). This source of resistance is not exploitable in durum wheat breeding due to its location on the D genome. As far as we know the genetic control of durum wheat resistance to WSSMV has never been reported and *Soldur* is the only durum wheat cultivar reported to be resistant. *Soldur* is therefore intensively used by breeders and identifying new sources of resistance could help to design a sustainable control of the WSSMV. The genetic architecture of the *Soldur* resistance to WSSMV has not been published yet.

This lack of diversity in the sources of resistance to WSSMV among the durum wheat cultivars is probably due to the severe bottlenecks experienced by durum wheat during the past millennia (Thuillet et al. 2005; Haudry et al. 2007). We thus broadened the search for WSSMV resistance by screening the diversity of wild (*Triticum turgidum dicoccoides*) and cultivated (*Triticum turgidum dicoccum*) emmers. This screening successfully identified accessions exhibiting a stable WSSMV resistance over years. Among these accessions, one, hereafter named *Dic2*, was used as a source of resistance for this study

As it is not yet possible to control WSSMV inoculation in glass-house or growth chambers, field experimentation is compulsory to score genetic material. Unfortunately WSSMV infected fields are frequently infected by related virus, such as SBCMV, which complicates the study of WSSMV specific resistance (Carroll et al. 1997; Vegetale 2003). Moreover, climate conditions greatly influence symptom severity observed on a given site that can range from no apparent symptoms to heavy damages on the plants (Cadle-Davidson et al. 2006). Lastly, spatial heterogeneity of virus repartition in the field makes sensitivity evaluation subject to large variations among repeats in the same site, sensitive genotypes being erroneously recorded as resistant when observed where soil inoculum is weak or absent (Cadle-Davidson et al. 2006). Thus, breeding for resistance to WSSMV based on phenotypic analysis is rather costly and slow. Marker-assisted selection (MAS) seems to be a promising approach for the pre-breeding of virus-resistant genitors from exotic germplasm.

In this study, we investigated the genetic basis of WSSMV resistance of *Dic2* via two RIL populations obtained by crossing the *Silur* and *Lloyd* elite cultivars with *Dic2*. RILs were phenotyped in 2012 and 2015, then genotyped using targeted locus capture (Holtz et al. 2016). We accounted for spatial heterogeneity of symptoms to improve RIL BLUP (Best Linear Unbiased Predictor) estimates. A consensus genetic map and a joint-QTL analysis lead to the identification and localization of major QTL of stable resistance to WSSMV.

**Material & Method**

**Plant Material and field trials**

Two F6 RIL populations were used in this study, both derived from a cross between a cultivated emmer wheat (*Triticum turgidum ssp dicoccum*) named *Dic2* and a durum elite variety (either *Silur* or *Lloyd*). The *Dic2* x *Silur* (DS) and *Dic2* x *Lloyd* (DL) mapping populations consisted of 161 and 186 recombinant inbreed lines (RILs) respectively. *Dic2* has been classified as resistant to WSSMV based on observations over multi-local and multi-annual field experimentations. In contrast, *Silur* and *Lloyd* are highly susceptible varieties.

All trials were carried out in Pray, a field with high WSSMV prevalence (Pray near Blois, in the central part of France, 47°40’27”N 1°07’58”E). The field shows a regular WSSMV infection and the absence of SBCMV is controlled by the absence of symptoms on the bread wheat Aztec cultivar known to be resistant to WSSMV and susceptible to SBCMV (M. Bonnefoy, pers. Com.). In very rare cases, symptomatic Aztec plant could be controlled positive by PCR to SBCMV using specific primers proposed by (Budge et al. 2008).

The two RIL populations were observed in 2012 and 2015. Corresponding experiments are hereafter called DS-2012, DL-2012, DS-2015 and DL-2015 (Table 1). Genotypes were grown following a randomized complete block design with 10 (resp. 34) lines and 75 (resp. 41) columns for 2012 (resp. 2015). For each genotype, several plants were grown in an observation unit consisting of two 1-meter-long adjacent rows manually seeded. Several genotypes were repeated twice for DS-2012, DS-2015 and DL-2015 (79, 54 and 54 repeated genotypes resp.), but no repetition was available for DL-2012. *Pescadou*, a highly susceptible check, was included regularly in the trials to control for spatial heterogeneity of the distribution using 149 phenotyped plants in 2012 and 100 phenotyped plants in 2015. The three parents were also included randomly in the trial (*Dic2*: 1 and 2 samples, *Silur*: 4 and 2 samples, *Lloyd*: 5 and 3 samples in 2012 and 2015 respectively). Experimental fields were sown in early October and managed following the agronomic practices commonly adopted in the area.

**Table 1**: Description of the four experiments: population name, number of RILs, number of repeated genotypes and phenotyping method used.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | DS-2012 |  | DL-2012 |  | DS-2015 |  | DL-2015 |
| Nb. genotypes | 165 |  | 186 |  | 164 |  | 189 |
| Nb. repeated genotypes | 79 |  | 0 |  | 54 |  | 54 |
| Visual assessment of Symptom Severity (SS) | Yes |  | Yes |  | Yes |  | Yes |
| ELISA | Yes |  | Yes |  | Yes |  | Yes |
| qPCR | No |  | No |  | Yes |  | Yes |

In 2013, DS and DL RILs were also sown at the DIASCOPE experimental station (Mauguio, South of France, 43°36'55"N 4°0'36"E) to record plant heights and flowering date.

**Genotyping**

F6 populations were genotyped in 2015 and used for QTL detection based on the 2012 and 2015 data. We used locus targeted genotyping following the protocol of (Holtz et al. 2016*)*. Briefly, two sets of 120 bp baits were designed to target 6240 and 10027 SNPs previously detected in the RNAseq data of *Dic2*, *Lloyd* and *Silur* genitors. RIL DNA was extracted in 2015 and captured following (Rohland and Reich 2012). Compared to (Holtz et al. 2016) blocking oligos were added to limit the capture of microsatellite-like sequences (Online Resource 1). Captured DNA was sequenced with two runs of HiSeq3000 (150 bp paired end reads). Reads were cleaned and mapped on a reference transcriptome of durum wheat (DWr) (Ranwez et al. 2013; David et al. 2014) using cutAdapt (Martin 2011) and bwa-mem (Li and Durbin 2009).

Putative chromosomal assignment and physical positions of the DWr contigs were obtained by blasting them on the bread wheat chromosome survey sequence for cv. Chinese Spring (BWr) generated by the IWGSC (Ensembl database release 28), (IWGSC 2014; Mayer et al. 2014; Chapman et al. 2015)). Genotypes were called using *Reads2snp* (Galtier et al. 2009) and SNPs were filtered according to the following criteria: i) inbreeding coefficients, *Fis*, above 0.8 corresponding to a low probability of being heterozygote, as 1.5% heterozygosity is expected on average after six successive selfing generations ii) at least 100 RILs genotyped for any given SNP, and iii) balanced frequencies with a minimum expected heterozygosity (Nei’s He, (Nei 1978)) of 0.34 so as to avoid strong segregation distortion, which is undesirable for genetic map building.

Four SSR markers known to be on the distal part of the chromosome 7B were also used to genotype the DS RILs: *Xbarc-1068*, *Xbarc-323*, *Gwm-400* and *Gwm-46* (http://wheat.pw.usda.gov/). These four markers are linked to SBCMV resistance (Maccaferri et al. 2011b). They were not used for the linkage map construction but positioned on it afterward. SNPs showing the highest linkage disequilibrium with those SSR markers were used to position them on our consensus genetic map. This allowed to test whether or not WSSMV and SBCMV resistance genes are collocated.

**Linkage map construction**

SNPs from the DS population (DS-SNPs) and from the DL population (DL-SNPs) were used to build two individual maps (DS-map and DL-map). The DS-map construction was described in (Holtz et al. 2016) with a focus on the capture technology. The DL-map has been constructed following the same procedure that is briefly recalled hereafter. Carthagene (de Givry et al. 2005) was used to assemble initial linkage groups (LGs) using a LOD score threshold of 7 (respectively 8 for DL) and a maximum two-point distance of 0.14 for DS (respectively 0.1 for DL). SNPs being already assigned to a BWr chromosome (best blast procedure (Holtz et al. 2016)), each LGs was then assigned to the most frequent carrier chromosome of its SNPs. Markers on LGs assigned to the same chromosome were pooled. Orders and distances between adjacent markers within chromosomes were finally determined using the *build*, *annealing*, *greedy* and *flips* algorithms implemented in Carthagene.

Then, common markers between DS-map and DL-map were used to build a consensus map containing all the markers using the Carthagene *dsmergen* function. Genetic maps were characterized and compared using the genetic map comparator web application (Holtz et al., submitted). Once positions of markers are set, missing data were imputed using the CallParentAllelesPlugin of Tassel (Glaubitz et al. 2014). Finally, we estimated the number of recombination events accumulated during the fixation of each RIL by counting the number of switches between stretches with successive parental allelic status.

**Phenotyping WSSMV resistance**

In 2012 and 2015, symptom severity (SS) was visually scored twice during periods exhibiting the maximum intensity of the disease symptoms. First notation (SS1) was performed on April 18th in 2012 and on March 27th in 2015, during Z32 stage of the Zadoks' scale of growth (Zadoks, Chang, and Konzak 1974). Second notation (SS2) was performed on May 15th in 2012 and April 14th in 2015, which respectively corresponded to stages Z49 and Z37 of Zadoks' scale. SS was scored using a 0-5 scale, where 0 represents no symptom and 5 a high mottling and stunting. Both within-year SS1 and SS2 were highly correlated, but SS2 notations were slightly more discriminant and better reflected WSSMV resistance (result not shown). We hence only consider SS2 from now on, which will be referred as SS.

Virus concentration was quantified using DAS-ELISA (Double Antibody Sandwich-Enzyme-Linked Immunosorbent Assay (Clark and Adams 1977)). For each observation unit, we randomly collected approx. 20 leaves at various developmental stages from different plants drawn at random. We ground 0.4 g of the pooled bundle of those leaves in 4 mL of a custom grinding buffer (Online Resource 2). We used the antiserum of (V. Marie-Jeanne A. Sohn 1999) provided by SEDIAG (WSSM-SRA, Bretenière, France, http://www.sediag.com/) and followed the manufacturer instructions except for the grinding buffer. A positive control was obtained using a pooled bundle of leaves collected on *Pescadou* plants with high SS scores and ground to give a single positive control extract aliquoted and frozen before use. Blank, negative and positive controls were included in each ELISA plate. Each sample was deposited in two successive pits on the plate. Absorbance was read at 405 nm in micro-plates reader (Tecan, infinite F200, Männedorf, Swiss). The Elisa score of each sample was quantified using the following formula:

Where *Elisa* is the quantitative Elisa score for a sample, *AbsS* is the absorbance of the sample, *blank* is the absorbance value of the blank of the Elisa plate, and *AbsPos* is the absorbance of the positive control of the plate.

Quantitative-PCR (qPCR) was performed on 2015 samples. The numbers of viral copies per mg of leaves were estimated as follows. RNA was extracted from 100 mg of the same leaf bundles used for ELISA. Purified RNA was then diluted 10 times and cDNA was produced by random hexamer priming. The real-time quantitative RT-PCR using the SYBR-Geen Chemistry follows the protocol described by (Vaïanopoulos et al. 2006) with some modifications. Briefly, amplification was conducted with diluted cDNA, the primers WSSMVc1-F and WSSMVc1-R using 40 PCR cycles (95°C, 15s, 60°C, 30s) on a Applied Biosystems StepOne Plus device. We use as DNA standard, a purified PCR product (WSSMVc2-F - WSSMVc1-R) covering a five points calibration range (104, 105, 106, 107, 108 copies) in the initial template (3 replicates per plate). A temperature range from 60°C to 95°C (0.3°C steps) was used for melting curves. Two technical replicates per cDNA were amplified*.* Numbers of viral copies per mg of leaves were obtained using the calibration. After all considerations, copy numbers are given for 22.8 µg of fresh leaves. All technical details are provided in Online Resource 2. Analyses were carried out on the log of copy numbers.

**Statistical analysis**

We partitioned the variance of each of the three resistance phenotypes (SS, Elisa and qPCR) into genetic and environmental variance components using the ASReml-R package in R (asreml 3.0, VSN International, Hempel Hemstead, UK, (Gilmour et al. 2009); R 3.3.2, <http://www.r-project.org/>, (R Development Core Team 2013)). The data was analyzed using the following full linear mixed model with a normal error distribution:

|  |  |
| --- | --- |
| , | (1) |

where is a vector of individual plant observations for a given trait; is a vector of fixed effects; is a vector of RIL random genetic effects; is a vector of random errors; and are incidence matrices relating the observations to the fixed and random effects respectively (Online Resource 3).

Fixed effects in for each trait comprised i) the overall mean of this trait, ii) two linear covariates to accommodate for possible linear environmental trends in virus concentration in the row and column directions, and iii) a type effect which accounts for average differences between the two RIL populations taken as a whole and the susceptible check (two levels: DL and DS RILs *vs*. Pescadou). For SS and Elisa only, fixed effects also comprised a year effect (two levels: 2012 or 2015) and an interaction between type and year effects. We used conditional Wald F-tests with a 5% significance level on the model with lowest AICc to test for fixed effects (Gilmour et al. 1997). The corrected Akaike Information Criterion (AICc) allows comparing models even when they are non-nested (AICc, Burnham and Anderson 2002).

For random genetic effects, variation in resistance across RILs was tested using different models for the distribution of : correlated genotypic effects between years (model A), uncorrelated genotypic effects between years (model B), constant genotypic effects between years (model C). For qPCR, a simple random genotype effect was estimated (model D). For environmental effects, heterogeneity in symptom spatial distribution was tested using several models assuming only spatially correlated errors (model 1, estimating a variance), only spatially uncorrelated errors (model 2, variance) or both types of errors (model 3, the error variance is partitioned between and ) (Gilmour et al. 1997).

Random effects were tested based on their AICc. Each model is ranked according to the difference between its AICc and the AICc of the model with lowest AICc. Following Burnham & Anderson (2002), we consider that models with are strongly supported by the data. The Best Linear Unbiased Predictor (BLUP) of each genotype was computed based on the model with lowest AICc and used for QTL analyses (see “QTL analyses” below). Individual heritabilities were computed for each trait using the estimates from the best model and their standard errors were computed using the delta method (Lynch and Walsh 1998). All details are provided in Online Resource 3.

**QTL analyses**

We used the QTLRel program (Cheng et al. 2011), implemented in R, for QTL analyses of the two DS and DL data sets. Sister lines were detected among our RILs after genotyping (27 in DS and 13 in DL), probably resulting from confusions during the fixation process in nurseries. Though these lines were removed from the map construction, we considered their phenotypes as worthy to increase the power for QTL detection as long as the lines relatedness was explicitly declared. QTLRel implements mixed models allowing a random polygenic effect using different kinship matrices taking into account the inherent relatedness among individuals (Cheng et al. 2010). We used the *GenMatrix* option of QTLRel, which uses the simple kinship coefficient matrix in the model and estimates it directly from marker data.

We performed a single marker analysis on BLUP values to compute a LOD score per marker. The QTL detection was first performed independently for each population and each year. A joint QTL analysis was then performed using both populations on markers found polymorphic in both DS and DL populations, but all markers were used to compute the kinship correlation matrix. The LOD threshold for declaring a QTL as significant for a trait in an experiment was obtained by permutations of genotypes relative to phenotypes. We kept as significance threshold the value of the 95% maximum LOD scores values obtained from 1000 independent permutations (Churchill and Doerge 1994). Since this threshold was always comprised between 3.24 and 3.61, a conservative LOD threshold of 3.61 was used for all traits. QTL confidence interval regions (in cM) were defined as the +/- 1.5 LOD-interval around the peak LOD values of each QTL (Mangin et al. 1994). Allelic effects were defined as half the difference between the BLUP means of favorable homozygous genotypes and the unfavorable ones.

All R scripts and data (\*\* for the reviewers: once the paper accepted) used for those statistical and QTL analyses are available in Online Resource 4 for reproducibility sake.

**Results**

**Genotyping and consensus linkage map constructions**

An average of 2.5 (resp. 4) million reads per sample was obtained for DS (resp. DL) and 86% (resp. 75%) of these reads were mapped successfully on DWr, the Durum Wheat reference transcriptome. After filtering on Fis, coverage and He, we retained 3734 SNPs for DS and 6887 SNPs for DL. Their parental allelic states were consistent with previous RNAseq data on these parents.

We assembled 22 and 31 linkage groups (LGs) for DS and DL respectively. Those assemblies contain respectively 14 and 16 large LGs (≥100 markers), which is consistent with the 14 chromosomes of durum wheat. The remaining LGs, though having fewer markers, were assigned to known chromosomes and assembled in the genetic maps. For DS, five markers were not linked to any other SNP and were not used in the genetic map (only one for DL). Finally, 3729 and 6886 markers build the two individual genetic maps for DS and DL respectively. The consensus genetic map contains 8568 markers with 2047 markers in common between both maps. Main features of the three maps are provided for each chromosome in Table 2, and mapping positions of individual markers are given in the Online Resource 5.

A majority (77%) of DL markers grouped in the same LG also have a common chromosomal putative assignment on BWr. A similar percentage (~80%) was observed in our previously published DS map (Holtz et al. 2016). In both cases, assignment inconsistencies were mainly due to homeologous competitive genome assignment (e.g. assignment to 1A instead of 1B). As putative assignment is determined by blast hits on BWr; a high degree of similarity between homeologous genes (or the absence of a homeologous copy in the reference) may lead to erroneous chromosome assignment. Translocations observed in the DS map were confirmed in the DL map. Indeed, 20 markers of the LG group representing chromosome 7A were initially putatively assigned by blast to chromosome 4A (reciprocally 15 on 4A initially assigned on 7A) and 14 markers of the chromosome 4B had putative assignment on chromosome 5A (reciprocally 14). This validates the translocation hypothesis proposed in (Holtz et al. 2016).

**Table 2. Features of the DS, DL and consensus durum wheat genetic maps.**

For each chromosome and each map the number of SNPs (#SNPs), total chromosome size in centiMorgan (Size (cM)), and the number of unique marker positions (unique pos.) are given. For the consensus genetic map, the average distance between two adjacent SNPs is also provided (Avg. inter marker distance=Size(cM)/#SNPs) as well as the Spearman’s rank correlation coefficients between this consensus genetic map and the putative physical positions (Spearman r with phys. pos.)

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | DS map (Dic2 x Silur) | | |  | DL map (Dic2 x Lloyd) | | |  | Consensus Map | | | | |
| Chr. |  | #SNPs | Size (cM) | unique pos. |  | #SNPs | Size (cM) | unique pos. |  | #SNPs | Size (cM) | unique pos. | Avg. inter marker distance | Spearman r with phys. pos. |
| 1A |  | 231 | 175.5 | 90 |  | 394 | 239.6 | 142 |  | 496 | 206.6 | 166 | 0.42 | 0.97 |
| 1B |  | 298 | 181.4 | 121 |  | 597 | 277.3 | 228 |  | 750 | 230.8 | 266 | 0.31 | 0.94 |
| 2A |  | 403 | 218.3 | 132 |  | 644 | 325.3 | 209 |  | 820 | 271.7 | 228 | 0.33 | 0.76 |
| 2B |  | 324 | 234.9 | 141 |  | 638 | 341.3 | 219 |  | 785 | 293.3 | 269 | 0.37 | 0.96 |
| 3A |  | 204 | 199.6 | 97 |  | 414 | 294.2 | 182 |  | 504 | 255.2 | 213 | 0.51 | 0.98 |
| 3B |  | 337 | 229.7 | 142 |  | 615 | 310.2 | 231 |  | 747 | 271.1 | 263 | 0.36 | 0.93 |
| 4A |  | 231 | 229.3 | 115 |  | 428 | 236.3 | 155 |  | 520 | 232.4 | 206 | 0.45 | 0.94 |
| 4B |  | 281 | 163.7 | 100 |  | 541 | 227.4 | 157 |  | 659 | 193.5 | 178 | 0.29 | 0.83 |
| 5A |  | 279 | 288.2 | 134 |  | 512 | 359.5 | 214 |  | 649 | 319.7 | 265 | 0.49 | 0.99 |
| 5B |  | 280 | 246.4 | 134 |  | 499 | 308.8 | 199 |  | 660 | 278.5 | 257 | 0.42 | 0.99 |
| 6A |  | 172 | 178.2 | 96 |  | 345 | 239.2 | 152 |  | 434 | 208.7 | 190 | 0.48 | 0.99 |
| 6B |  | 253 | 183.4 | 114 |  | 483 | 241 | 176 |  | 564 | 213.5 | 205 | 0.38 | 0.94 |
| 7A |  | 292 | 237.6 | 135 |  | 451 | 334.2 | 185 |  | 587 | 285.4 | 228 | 0.49 | 0.99 |
| 7B |  | 144 | 197.3 | 73 |  | 325 | 253.2 | 139 |  | 393 | 227.7 | 160 | 0.58 | 0.96 |
| Mean |  | 266.4 | 211.7 | 116.0 |  | 491.9 | 284.8 | 184.9 |  | 612.0 | 249.2 | 221.0 | 0.42 | 0.94 |
| Total |  | 3729 | 2964 | 1624 |  | 6886 | 3988 | 2588 |  | 8568 | 3488 | 3094 |  |  |

The DL map is 1.35 time longer than the DS map, with 3988 cM (for 6886 SNPs) versus 2964 cM (for 3729 SNPs). The consensus map length is 3488 cM (for 8568 SNPs) and well resolved with a high number of markers per chromosome (average of 612, ranging from 393 for chromosome 7B to 820 for chromosome 2A). The average inter-marker genetic distance is 0.42 cM and ranges from 0.29 (chromosome 4B) to 0.58 cM (chromosome 7B). The longest gap per chromosome is 12 cM long on average, but is always smaller than 20.3 cM (chromosome 1A). This consensus map contains 3094 unique positions, well spread among and along chromosomes. The marker repartition along the three maps and the colinearity between markers of the three maps are represented in Fig. 1 for the chromosome 7B, and in Online Resource 6 for all other chromosomes. These figures illustrate the high density of the three maps as well as their consistency.

**Fig. 1: Consensus and individual map representation.**

The three genetic maps of the chromosome 7B: DL map on the left, DS map on the right and the consensus map in the middle. Common markers of adjacent maps are linked by blue lines.

The genetic ordering of the SNPs within the consensus map is highly consistent with the physical positions of their contig in the BWr. Indeed, the Spearman’s rank correlations between those two SNP orders are 0.9 on average (min 0.76 for chromosome 2A, max 0.99 for 5A). The four SSR markers are linked to SNP markers of the 7B chromosome, at positions 0.0, 5.3, 27.6, and 54.7 cM for Xbarc-1068, Xbarc-323, Gwm-400 and Gwm-46 respectively.

**Evaluation of WSSMV resistance**

Detail values for the selection of the best models according to their AICc and likelihoods are reported in tables of Online Resource 8.

*Phenotypic variation*

No significant year effect was observed except on *Pescadou.* Genitors of the two RIL populations presented the expected phenotype: *Dic2*, the resistant genitor, showed a high resistance (but not immunity. Elite genitors *Silur* and *Lloyd* were highly affected both years, with mean SS ranging from 4.1 to 4.7. The DS and DL RIL populations showed a large segregation, with accessions WSSMV sensitivity ranging from very low (SS~0) to very high (SS~5) (Table 3).

**Table 3: Features of the four trials**

Mean visual assessment of symptom severity (SS) and virus concentration (ELISA absorbance value and qPCR value (logarithm of virus copy number per 22.8 µg of fresh leaves) for the two recombinant inbred lines (RILs) populations evaluated in field trials in 2012 and 2015. Measures range (minimum and maximum), phenotypic coefficient of variation (CV) and broad sense heritability values (H²) are reported.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | DS 2012 | |  | DL 2012 | |  | DS 2015 | | |  | DL 2015 | | |
|  | SS | ELISA |  | SS | ELISA |  | SS | ELISA | qPCR |  | SS | ELISA | qPCR |
| Mean | 2.01 | 0.87 |  | 2.28 | 0.87 |  | 2.07 | 0.62 | 8.83 |  | 2.48 | 0.72 | 10.06 |
| Min | 0.00 | 0.04 |  | 0.00 | 0.03 |  | 0.00 | 0.01 | -4.57 |  | 0.00 | 0.01 | -1.98 |
| Max | 5.00 | 2.76 |  | 4.50 | 2.39 |  | 5.00 | 1.35 | 17.55 |  | 5.00 | 1.39 | 18.45 |
| CV | 0.51 | 0.56 |  | 0.48 | 0.54 |  | 0.66 | 0.71 | 56.90 |  | 0.56 | 0.59 | 49.87 |
| H2 | 0.65 | 0.41 |  | - | - |  | 0.64 | 0.78 | 0.62 |  | 0.64 | 0.77 | 0.62 |

Pearson coefficient of correlation between SS and ELISA infection evaluations were 0.53 for 2012 and 0.66 for 2015. ELISA and qPCR correlations were high (0.72). This denotes a slight discrepancy between the three methods of WSSMV infection evaluation. The heritability of ELISA was higher than for SS (Table 3) in 2015, but lower in 2012.

*Spatial heterogeneity of WSSMV infection*

For all traits, model 3 (the environmental error being partly spatially correlated and uncorrelated) always had the lowest AICc (Table S1A, S2A and S3A respectively). The two variance components, and had similar order of magnitude within year (e.g., Elisa 2012, = 0.1; = 0.12, Elisa 2015, = 0.02; = 0.04, Table S2D). This suggests that virus infection was not uniform within the experimental field. In addition, the best models consistently included spatial auto-correlation both in the row and column directions of the field. The column auto-correlation parameter, , was always higher than the row auto-correlation parameter, , (e.g., = 1, = 0.47, Elisa 2012, Table S2D). This difference is consistent with smaller distances (and hence higher correlation) between adjacent columns (20 cm apart) than adjacent rows (1.5 m apart). As no significant general row and column effects were detected, this heterogeneity was fine grained and patchy (Table S1B and S2B). For qPCR nevertheless, models 3 and model 1 (without spatial autocorrelation) had similar AICc values (Table S3A) but model 3 had a slightly higher likelihood. This is likely due to the fewer number of observations in this dataset. Furthermore, we detected a trend similar for and to the one observed on SS and ELISA.

For random genetic effects on SS or ELISA, model A (correlated genotypic effects between years) always had the lowest AICc (Table S1A and S2A respectively). Genetic variances were consistently higher in 2015 compared to 2012 (e.g. for ELISA, 0.07 *vs*. 0.13 in DL and 0.08 *vs*. 0.14 in DS, Table S2D). ELISA heritabilities were higher in 2015 (0.78 in DS and 0.77 in DL) than in 2012 (0.41 in DS and 0.41 in DL), whereas SS heritabilities were similar for the two years and populations (0.65 for both DS and DL, Table 3). This indicates that, in 2015, the genetic variance in viral load increased relatively more than , whereas genetic variance in SS increased by the same order of magnitude as . For qPCR, heritabilities were equal between the two RIL populations (0.62 for both DS and DL, Table 3). For SS and ELISA, genetic correlations between 2012 and 2015 were positive and consistently higher than 0.6 (e.g. for ELISA, 0.78 for both DL and DS, Table S2D). As they are very similar to the heritabilities computed for each year, Genotype by Year interactions were likely to be limited.

*Resistance: relationship with plant height and earliness*

Phenotypic correlations between WSSMV resistance and plant height or precocity were always very low. Pearson correlation between SS, ELISA and qPCR BLUPs and heading or earliness were comprised between 0.02 and 0.14 for DS, or between -0.01 and 0.03 for DL. There was no correlation between WSSMV resistance and plant height (Pearson correlation in range of [-0.24; -0.15] for DS, and [-0.12; 0.09] for DL). This suggests that neither height nor earliness affects WSSMV resistance.

**Genetic control of WSSMV resistance**

The joint QTL detection combined the 161 and 186 RILs of DS and DL populations genotyped for 2054 common SNPs. Features of the significant QTL are summarized population by population in Online Resource 9 and located on our consensus genetic map in Fig. 2.

A first major QTL, hereafter Qssm-mtp-7BS, was detected in the distal area of the short arm of chromosome 7B. The second one was observed on chromosome 7A in the sub centromeric area, hereafter Qssm-mtp-7A. All WSSMV resistance traits were partly explained by these QTL for both years (Fig 3).

For Qssm-mtp-7BS, LOD scores ranged from 5.32 (SS in 2012) to 8.69 (ELISA in 2012). This QTL explained between 5.79% and 9.56% of the total phenotypic variation. For all phenotypic traits, the LOD scores are highly congruent. The +/- 1.5 LOD score confidence interval pinpoints to a locus spanning ~5cM [54.4 to 59.3 cM] on chromosome 7B. This confidence interval contains 10 SNPs. Eight of them are spread on a putative physical zone ranging from 36.6 to 56 Mb, the two remaining distal markers being located at 111 and 142 Mb in the BWr.

For Qssm-mtp-7A, LOD scores ranged from 5 (SS 2012) to 9.5 (ELISA in 2012). This QTL explained between 5.42% and 10.42% of the total phenotypic variation. For all phenotypic traits, the LOD scores are highly congruent and pinpoint to a small area of 3.9 cM [115.5 to 119.4 cM]. The confidence interval contains 11 SNP markers distributed in a putative physical zone ranging from 43.7 to 55 Mb.

A minor QTL (Qssm-mtp-2AL) was detected on the long arm of chromosome 2A for SS 2015, SS 2012 and ELISA 2015. LOD-scores ranged from 3.61 to 5.82 and explained from 3.8% to 6.29% of the BLUP variance. The QTL lies in the [214.7-227.1] cM confidence interval containing 18 markers covering a relatively short putative physical area ranging from 237.1 to 241.3 Mb

These QTL were also detected on individual maps (Fig 2) but with larger confidence intervals (Online resource 9) and no population specific QTL was detected. This denotes a very high consistency of these QTL between years, populations and phenotyping methods. The favorable allele conferring resistance was inherited from *Dic2* in all cases.

**Fig. 2: Consensus genetic map and detected QTL**

Chromosome 2A, 7A and 7B are represented by three lines of grey points. Each point is a marker of the consensus genetic map. The genetic scale (in cM) is provided on the left side. Locations of QTL confidence intervals are indicated by colored lines: Light (2012) and dark (2015) blue lines for SS; light (2012) and dark (2015) greens lines for ELISA, and pink for qPCR (2015). Colored lines on left of the chromosome depict QTL found within the DS population, while colored lines on the right depict QTL identified within the DL population.

**Fig. 3: LOD-scores for markers on chromosome 7A and 7B**

LOD scores for association with WSSMV resistance detected by simple interval mapping with QTL-Rel are represented for every marker of chromosomes 7A and 7B. To improve readability, the points representing LOD scores of each phenotypic trait are linked by colored lines (following the color convention used in Fig 2).

*Epistatic interaction between the two major QTL*

Interaction effects between Qssm-mtp-7BS and Qssm-mtp-7A were tested using a simple linear model declaring additive and interaction effects by pair of markers. To limit the combinatorial inflation, and the associate computation time growth, each QTL was represented by its best marker (i.e., having the peak LOD score in the single marker analysis). A significant (*pvalue* < 10-6) interaction was observed for all trials and resistance phenotypes (Online resource 9). For example for ELISA 2015, the actual difference between the double resistant R7A-R7B andthe double susceptible S7A-S7B was -0.53 while the difference between the values predicted by additive effects was -0.25 only (with a7A=-0.075 and a7B=-0.06~~,~~ Online Ressource 9). The plants having a single resistance allele are almost as sensible as those having none, while the plants carrying both the favorable alleles at Qssm-mtp-7BS and Qssm-mtp-7A are highly resistant (Fig. 4). First and second order interactions implying Qssm-mtp-2A were not significant (result not shown).

**Fig. 4: Epistatic interaction between Qssm-mtp-7BS and Qssm-mtp-7A QTL for ELISA in 2015 on 345 RILs**

Let’s denote by R7A (resp. R7B) the resistant allele at Qssm-mtp-7A (resp. Qssm-mtp-7BS) and S7A (resp. S7B) the corresponding sensitive allele. Individual BLUP value distributions are summarized by box-plots for R7A-R7B, R7A-S7B, S7A-R7B, S7A-S7B haplotypes. The plants having a single resistance allele are almost as sensible as those having none, while the plants carrying both the favorable alleles at Qssm-mtp-7BS and Qssm-mtp-7A are highly resistant.

**Discussion**

**Locus targeted capture: high efficiency for genotyping and map building**

In a previous study, we demonstrated that genotyping by capture is well suited for durum wheat genotyping and genetic map construction (Holtz et al. 2016)*.* For the DL map, important improvements were carried out: only two baits per SNP were designed for a greater productivity, SNPs within or nearby microsatellite-like regions were not used for bait design and blocking oligos were used during the capture phase. Taken as a whole these measures significantly decreased the undesired capture of repeated sequences and allowed to almost double the number of useful SNPs that passed our quality check filters: from 3729 for DS to 6886 for DL, i.e., a 84% increase of productivity. The resulting DL map contains 2588 unique positions, more than most of the so far published durum wheat maps, including those built with the 90K wheat array (Maccaferri et al. 2014).

Our DS and DL maps share 2054 markers, i.e. 55% of the DS markers and 30% of the DL ones. The genetic orders of the common markers are in strong agreement in the two maps: the Spearman’s rank correlation between both maps orders along the 14 chromosomes of durum wheat ranges from 0.99 to 1. This allowed us to build a highly reliable consensus map containing 3094 unique positions with 8568 markers. This consensus map shows strong colinearity (Spearman correlation > 0.92) with the bread wheat reference (except for chromosomes 2A (Spearman correlation 0.76) and 4B (Spearman correlation 0.84). This consensus map is thus a highly valuable tool for QTL mapping in durum wheat. The expected publication of the durum wheat genome (Distelfeld 2016) would be an invaluable resource to ascertain the physical position of the markers presented here.

Our consensus genetic map (3488 cM) is longer than other durum or cultivated emmer maps (2,635 cM, (Maccaferri et al. 2014)), and SNP-based maps of wild wheat (2,258 cM, (Avni et al. 2014)). This kind of difference between genetic map length in durum wheat has already been reported (Leflon et al. 2010; Vaissayre et al. 2012). Here, map increase in length should come from a high *per se* recombination rate of the Lloyd cultivar which has already been detected in a previous study (Vaissayre et al. 2012). Variation in recombination rate among genotypes has also be documented and tested in other species (Esch et al. 2007). We observed that the number of crossing-overs was significantly higher in DL than in DS RILs (68 vs. 50.3 crossing-overs on average per RIL, *P* <10-8).

The DL map and proposed physical assignments in BWr presents the same chromosome assignment discrepancies as those previously documented between the DS map and BWr assignments (Holtz et al. 2016). This confirms that durum wheat has some translocations between chromosome 4B and 5A, and between chromosomes 4A and 7A, that may be different than those of bread wheat.

**Phenotyping the WSSMV resistance of durum wheat**

We observed some within year inconsistencies between the phenotyping methods of the same sample. Several explanations can be proposed: i/ SS evaluation can be biased if WSSMV symptoms are confounded with other symptoms, e.g., due to possible rare spots of SBCMV; ii/ ELISA test is based on an extract of 0.4 g from a bundle (ca. 20) of randomly drawn leaves while the expert can spot one individual plant with symptoms among 30 to 40 plants to score SS; iii/ symptomless carriers may exist if some plants/genotypes have a WSSMV tolerance (Carroll et al. 1997). Though qPCR is described as a very sensitive (and expensive) method (Vaïanopoulos et al. 2006), it could even be more dependent on the sampling procedure than ELISA since only 0.1 g of leaves are sampled to represent the two rows of an experimental unit. Indeed, qPCR yielded a lower heritability in our study than SS and ELISA. This confirms that sampling is a crucial point for virus evaluation, especially when the attack is mild, as it may be the case in Pray. Another procedure could be envisaged for ELISA and qPCR: harvesting, pooling and grinding a large quantity of leaves and then sampling the extracted juice but this would be almost untractable and very expensive for such large number of analysis. Evaluating WSSMV resistance with SS and ELISA appeared to be the best compromise.

Spatial autocorrelation in symptoms confirms some previous studies (Cadle-Davidson et al. 2006), indicating that soil virus concentration may be highly variable at the field level or that some environmental factor may modify locally the virulence of the virus (such as local immersion of roots). Our model describing spatial heterogeneity permitted to get improved accuracy in RIL BLUP values and therefore to increase the LOD scores at the peak values in the QTL detection (up to 1.5 LOD in the better cases, data not shown). This increased accuracy led to smaller confidence intervals around the markers showing the LOD peak values.

If WSSMV symptoms expression could vary based on climatic conditions (Cadle-Davidson et al. 2006), our data suggest that the ranking of genotype resistance of our lines was very stable, since the “between year genetic correlation” was high compared to the trait heritability. This was also confirmed by the very consistent detection of QTL among populations, years and phenotyping methods.

**Role of the homeologous group 7 in virus resistance**

Two main QTL for WSSMV resistance were found on chromosomes 7A and 7B. These QTL were detected consistently through years, phenotyping methods and populations. The joint analysis of the two RIL populations permitted to reach LOD scores over 8 and to reduce the confidence interval to 4.2 and 4.9 cM for QTL 7A and 7B respectively. These two QTL are not in homeologous positions (56 cM on average on 7B and 116 cM on the 7A). Their underlying causal genes may thus not be linked to the same resistance mechanisms. Another QTL, Qsbm-ubo-7BS, has been reported on the chromosome 7B in durum wheat for SBCMV resistance positionned between markers Xbarc-1068 and Gwm-400 (Maccaferri et al. 2011b) or below Gwm-400 (Maccaferri et al. 2011a; Russo et al. 2012). This region is projected between 0 and 27.6 cM in our high-resolution consensus genetic map, suggesting that Qssm-Mtp-7BS is different from Qsbm-ubo-7BS but further investigations are needed to sort out this question. Another minor QTL concerning the WYMV resistance of bread wheat has been described by (Zhu et al. 2012) on the chromosome 7B just nearby the Gwm-46 marker. This marker is located at 54.7 cM in our consensus genetic map, colocalizing near the Qssm-Mtp-7BS. A cluster of expressed resistance genes (Rgenes) have been also reported on homeologous group 7 (Dilbirligi et al. 2004).

**Role of chromosomes 2**

In our study, a QTL (Qssm-mtp-2AL) was detected in the sub telomeric region of the long arm of the chromosome 2A (227 cM). Other QTL for the resistance to mosaic viruses have been reported in the literature on the homeologous group 2: a distal QTL on the short arm of chromosome 2B (6-18 cM, QSbm.ubo-2BS) for SBCMV resistance in durum wheat (Maccaferri et al. 2011b; Russo et al. 2012), a distal QTL in bread wheat for WYMV (Qym1) on the short arm chromosome 2D (60 cM) (Suzuki et al, 2015), and a QTL for bread wheat resistance to WSSMV on the long arm of chromosome 2D (Khan et al 2010). Even if we could not ascertain colocalization as for the QTL in chromosomes 7, it is very unlikely that the genes underlying the above mentioned QTL are homeologous.

For A and B genomes, homeologous groups 2 and 7 had the highest number of well annotated Rgenes on the bread wheat sequence (Bouktila et al. 2015) but much deeper investigations are still needed to verify the virus resistance are determined by Rgenes.

**Epistatic interactions**

A synergistic effect exists between the resistance alleles at the 7A and 7B QTL, which denotes an epistatic positive interaction. When considered separately, each of these two QTL explains between 5 and 9% of the phenotypic variance only, but between 22 and 43% when considered together. On average, plants having the two resistant alleles have a much stronger resistance than plants having one or no favorable allele. Such a complex genetic control has already been observed for two other virus transmitted by *Polymyxa graminis* (Walker et al. 1998; Gutiérrez et al. 2010). On WYMV, a bymovirus relatively close to WSSMV (Liu et al.), the Qym1 and Qym2 locus seem also to have a positive interaction effect, resistant alleles at the two locus being necessary to get a high level of resistance (Suzuki et al. 2015).

The genetic determinism of virus resistance appears thus quite complex and many different chromosomal regions may be involved in the interaction with the viruses. If such epistasis interactions were to be confirmed on a larger number of situations, this could lead to very different QTL effect estimates according to the polymorphism between the two parents. If the two parents of a cross share a common allele at one locus, allelic effects detected at the other locus may be very different: from a strong additive effect if the parents are fixed for the resistant allele to no significant (or weak) effect if they are fixed for the susceptible allele. Hence *durum* x *durum* crosses may reveal mostly additive effects at Qtls while *dicoccum* x *durum* crosses may yield more complex situations. We do not know yet if the genetic determinism of the WSSMV resistance described in *Dic2* is different from that of *Soldur* and more investigation are needed to clarify how the virus resistance has been build and modified during the durum wheat history.

**Mechanisms of resistance**

Two bymovirus (WSSMV and WYMV) are infectious on wheat and induce rather similar symptoms (Clover and Henry 1999). Though molecularly not very close (i.e., 70% of identity on their coat protein (Liu et al.)), mechanisms of resistance may share some similarities. In bread wheat, WYMV resistance is not based on the prevention of the root colonization by *P. graminis* but based on the inhibition of viral multiplication in the root cells or viral transmission among them (Liu et al.). As our WSSMV QTL detected here do not colocalize with *Qym1*, *Qym2* nor *Qym3* (Suzuki et al. 2015) the molecular basis of resistance may be different and the mechanism of resistance of *Dic2* and *Soldur* still have to be explored, notably by testing the different genotypic combinations of the three QTL for their ability to control the virus multiplication in the roots or its transmission to the leaves.

The existence of strong epistatic positive interaction between *Qssm-mtp-7A* and *Qssm-mtp-7BS* may suggest that two successive steps are at stake or that two genes are necessary to control one component of resistance. As the two major QTL are located in medium recombining areas, much work remains to be done to develop markers closer to the causal genes and further to clone the underlying causal genes.

XXX Véronique (MarieJeanne) : Peux tu voir si la discussion peut être approfondie virologiquement parlant

**Plant breeding and WSSMV resistance**

The difficulty and cost of acquiring WSSMV phenotypic values emphasize the interest of a molecular breeding approach. Marker assisted selection (MAS) is all the more promising as the QTL in this study are consistently detected for different years and populations, and thus seem to be very good proxy for up to 40% of the WSSMV resistance variation. KASPAR markers could be easily developed from the sequence information provided in this paper and used routinely in breeding program.

The two major QTL should be selected together which may transfer a genetic burden to bred lines if the *Dic2* emmer carries some unfavorable alleles close to these locus. However QTL are not located close to major agronomic locus such as Rht1 locus (4BS, (Gale and Marshall 1976)) or Vrn locus (Yan et al. 2004). Furthermore, WSSMV resistance did not correlate with plant height and flowering date. So we have good hope that these two Q*Swm.Mtp* can be mobilized in breeding program with little detrimental effect on the agronomical value.

We do not know yet if the genetic determinism of the WSSMV resistance of *Dic2* is different from that of *Soldur*. If resistance genes revealed to be different, which is possible since emmer and durum wheat have many specific alleles, the use of both resistance sources would provide an opportunity to better manage resistance sustainability.

**Author’s contributions**

YH analyzed the data, wrote most of the paper and coordinate the writing process. MB supervised the field experiment and performed the visual phenotyping. VV performed the ELISA and qPCR analysis. MA produced the DNA libraries and produced the genomic data. GP and VV created and maintained the RILs. VV, VR, NR and PR were involved in discussions concerning data production and analysis; they also participate to the writing process. SS supervised the bio molecular work. DG and JD initiated the project. JD proposed the method, supervised the work and was involved in the writing. All authors read and approved the current version of the paper.

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# Supporting Information

**Online Resource 1: Genotyping by capture protocol**

Description of the protocol used to capture DNA using the myBait technology with specific oligos.

**Online Resource 2: Protocol used for Elisa and qPCR**

Description of the protocol used to phenotype leaf samples with Elisa and qPCR.

**Online Resource 3: Data and R scripts for a reproducible QTL detection**

Data and R script (.csv and .rmd format) are provided in this tar archive. A scheme aims to explain the content of each file and its role in the QTL detection pipeline. The upstream bioinformatics steps (from raw reads to consensus genetic map) are not included.

**Online Resource 4: Genetic maps**

This excel file contains 3 sheets giving the information concerning i) the consensus genetic maps, ii) the DS map and iii) the DL map. The information is organized as an array with 3 fields in a row per marker: the chromosome assigned to this marker, the marker name, and the marker positions (in cM) within the chromosome.

**Online Resource 5: Visualization of the Genetic maps**

For each chromosome, 3 parallel black lines represent the 3 genetic maps (DL, consensus and DS) with lengths represented in cM. The consensus map is represented in the middle, with the DS map on its left and the DL map on its right. Each marker is represented by a black point indicating its position along the chromosome. Blue lines link common markers between 2 adjacent maps.

**Online Resource 6: Observation of the spatial heterogeneity of WSSMV infection**

Experiments of 2012 and 2015 are represented in 2 distinct sheets. Each cell represents an accession. The color of the cell reflects the mean Symptom Severity observed in the direct neighborhood of the corresponding accession (including itself). Red indicates a strong infection (SS=5) and white indicates no infection (SS=0).

**Online Resource 7: Model selection using AICc**

This file gives details concerning the selection of the model used for STL detection. It provides, for each trait (SS, Elisa and qPCR), the AICc and main features of every tested models.

**Online Resource 8: Detail of every QTL detected**

Here are reported details of every QTL detected with simple interval mapping analysis. Meta- QTL are first reported, followed by QTL of the DS and DL RILs. For each QTL we reported: LOD score, position and name of the marker with the highest LOD, LOD-1.5 confidence interval, additive effect. This latter is here defined as half of the difference between the mean value of the RILs carrying the susceptible allele (*Silur* and *Lloyd*) and the RILs with the resistant allele (*Dic2*). SS denotes symptom severity.

**Online Resource 9: Visualization of QTL along chromosome 7A and 7B for DS and DL**

Four graphics are provided that depict LOD scores observed for DS (two graphics on top) or DL (two graphic on bottom) along chromosome 7A (left graphics) or 7B (right). In each graphic, LOD scores for association with WSSMV resistance detected by simple interval mapping with QTL-Rel are represented for every marker. The LOD scores of each phenotypic variable (SS, ELISA and qPCR in 2012 and 2015) is represented by a specific color.

**Online Resource 10: Description of interaction between 7A-QTL and 7B-QTL**

For each phenotypic trait having both 7A and 7B significant QTL, (SS-2012, SS-2015, Elisa-2012, Elisa-2015), the distribution of the BLUP values of each resistance haplotype (R7A-R7B, R7A-S7B, S7A-R7B, S7A-S7B) is summarized by a boxplot. The interaction between both QTL has been tested using a simple linear model and was always highly significant.