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ORIGINAL PAPER

Resistance to *Soil-borne cereal mosaic virus* in durum wheat is controlled by a major QTL on chromosome arm 2BS and minor loci

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Abstract *Soil-borne cereal mosaic* (SBCM) is a viral disease, which seriously affects hexaploid as well as tetraploid wheat crops in Europe. In durum wheat (*Triticum durum* Desf.), the elite germplasm is characterized by a wide range of responses to SBCMV, from susceptibility to almost complete resistance. In this study, the genetic analysis of SBCMV resistance was carried out using a population of 181 durum wheat recombinant inbred lines (RILs) obtained from Meridiano (resistant) × Claudio (moderately susceptible), which were profiled with SSR and DArT markers. The RILs were characterized for SBCMV response in the field under severe and uniform SBCMV infection during 2007 and 2008. A wide range of disease reactions (as estimated by symptom severity and DAS-ELISA) was observed. A large portion of the variability for SBCMV response was explained by a major QTL (*QSbm.ubo-2BS*) located in the distal telomeric region of chromosome 2BS near the marker triplet *Xbarc35–Xwmc661–Xgwm210*, with R^2 values ranging from 51.6 to 91.6%. The favorable allele was contributed by Meridiano. Several QTLs with minor effects on SBCMV response were also detected. Consistently with the observed transgressive segregation, the resistance alleles at minor QTLs were contributed by both parents. The presence and effects of *QSbm.ubo-2BS* were validated through association mapping in a panel of 111 elite durum wheat accessions.

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Introduction

Soil-borne cereal mosaic virus (SBCMV), *Soil-borne wheat mosaic virus* (SBWMV) and *Chinese wheat mosaic virus* (CWMV) are closely related furoviruses (Torrance and Koenig 2005; Shirako et al. 2000) that greatly affect the production of durum (*Triticum durum* Desf.) and common wheat (*T. aestivum* L.; McKinney 1923; Kucharek and Walker 1974; Bonnefoy et al. 1994; Rubies-Autonell et al. 2003, 2009; Budge et al. 2008b). These viruses are transmitted to the roots of their hosts by *Polymyxa graminis* Led., a soil-borne plasmodiophorid capable of preserving the soil-borne virus infectivity in the soil for 10 years or longer (Canova 1966; Estes and Brakke 1966; Kanyuka et al. 2003).

SBCMV is widespread in Europe, CWMV in Asia and SBWMV, the furovirus-type member, in North America (Jesewska 1994; Clover et al. 1999; Diao et al. 1999; Ratti et al. 2005; Kühne 2009; Vaïanopoulos et al. 2009). In Italy, SBCMV is widespread in the main durum and common wheat-growing areas, especially in the northern and central regions, where it often causes grain yield losses up to 50% or more on susceptible cultivars (cvs.) as reported by Vallega and Rubies Autonell (1985); Vallega et al. (1999); Rubies et al. (2003, 2009). To date, the

development of resistant cvs. represents the main strategy to control these viruses (Bass et al. 2006; Perovic et al. 2009).

The genetic control of resistance in common wheat ($2n = 6x = 42$; AABBDD) has been studied extensively. Resistance was found to be controlled by one to three genes or major quantitative trait loci (QTLs; Nakagawa et al. 1959; Shaalan et al. 1966; Dubey et al. 1970; Modawi et al. 1982; Merkle and Smith 1983; Barbosa et al. 2001; Bass et al. 2006; Narasimhamoorthy et al. 2006; Hall et al. 2009). Two major loci for resistant response have been mapped: *Sbm1* in the distal end of chromosome (chr.) 5DL, which has been shown effective against SBWMV in the USA (Narasimhamoorthy et al. 2006) and SBCMV in Europe (Bass et al. 2006), and *Sbm2* (short arm of chr. 2B), against SBCMV in Europe (Bayles et al. 2007). Thus, according to the literature, resistance to SBCMV in common wheat appears to be controlled by a few major genes.

Genetic mapping studies on the response of durum wheat ($2n = 4x = 28$; AABB) to furoviruses have not yet been published (only preliminary results have been reported by Maccaferri et al. 2008c). Field trials carried out in Italy using nearly 200 cvs. of various origin evidenced a wide and continuous range of reactions to SBCMV, strongly suggesting that SBCMV resistance in durum wheat was under polygenic or oligogenic control (Vallega and Rubies Autonell 1985; Vallega 2004; Ratti et al. 2006; Rubies-Autonell et al. 2009). Moreover, very few of the cvs. showed extreme reactions and none proved immune to SBCMV (Vallega 2004; Rubies-Autonell et al. 2009).

Molecular tools necessary to identify the loci governing the traits of interest are now available (Varshney and Tuberosa 2007). In hexaploid wheat, numerous microsatellite markers (simple sequence repeat loci, SSRs) have been developed and used to construct linkage maps (Röder et al. 1998; Somers et al. 2004; Song et al. 2005). Hence, genetic mapping and QTL analysis in durum wheat can be carried out using the molecular markers developed for the A and B genomes of hexaploid wheat (Korzun et al. 1999; Eujayl et al. 2002; Maccaferri et al. 2003). The SSR-based maps can be further saturated with high-throughput markers, such as those based on microarray-hybridization assays (DArT® markers, Mantovani et al. 2008).

Bi-parental recombinant inbred mapping populations have been developed and characterized in *T. durum* to enable the identification of chr. regions harboring genes/QTLs for target traits (Blanco et al. 1998; Nachit et al. 2001; Maccaferri et al. 2008a, b). Association mapping (AM), based on the molecular and phenotypic characterization of germplasm collections, is increasingly being utilized in many crop species as a method complementary to traditional bi-parental linkage mapping (Ersoz et al. 2007). AM is based on the recombination events cumulated over generations, quantified by means of linkage-disequilibrium (LD) estimates. LD in collections of elite accessions of self-pollinating cereals has been observed to extend over several cM (long-range LD), typically from 5 to 10 cM in the case of common and durum wheat (Maccaferri et al. 2005, 2006; Breseghello and Sorrells 2006; Chao et al. 2007; Comadran et al. 2009).

In this study, we investigated the genetic basis of the inheritance of field response to SBCMV using both bi-parental linkage mapping and AM. The genetic determinants of the resistance carried by the Italian elite durum wheat cv. Meridiano, which is characterized by a high degree of field resistance to SBCMV (Rubies-Autonell et al. 2009), were investigated in a recombinant inbred line (RIL) population obtained from the cross between Meridiano and Claudio, a moderately susceptible cultivar. The main genetic determinant found in the RIL population was validated by means of AM using the SBCMV-response data of an elite durum germplasm collection.

Materials and methods

Plant materials

A durum wheat population of 181 RILs ($F_{7:8}$), developed by Produttori Sementi Bologna SpA (Argelato, Italy) following the single-seed descent method from the cross Meridiano × Claudio (M × C) was used. Both parents were elite Italian cvs. obtained from complex crosses among Italian, CIMMYT and North American accessions. Under the Mediterranean growing conditions, Meridiano and Claudio are medium-early and medium-late heading cvs., respectively, with high grain yield potential across southern Europe. Meridiano (pedigree: Simeto/WB881//Duilio/F21) is resistant to SBCMV, whereas Claudio (pedigree: CIMMYT selection/Durango//ISI938/Grazia) is characterized by a moderate susceptibility (Rubies-Autonell et al. 2009).

A panel of 111 elite accessions was used to further investigate, through AM, the presence and the effect of the major QTL for SBCMV response found in the M × C population. The panel includes accessions of the elite germplasm from the Mediterranean basin and North America. The origin and the genetic structure of the durum accessions have been described in Maccaferri et al. (2005) and their SBCMV response in Ratti et al. (2006).

Field trials

The trials were carried out near Bologna, northern Italy (Po Valley), in fields characterized by a severe and uniform SBCMV infection: the association panel was evaluated near Minerbio (44°37′ N 11°29′ E) in 2003 and 2004 (Ratti

et al. 2006), whereas the RIL population was evaluated near Cadriano (44°35′ N 11°27′ E) for two consecutive seasons (2007 and 2008).

A randomized complete block design with two replicates was adopted in all trials. The association panel was evaluated in plots consisting of three 1.25 m-long solid-seeded rows. Twelve plots with the highly susceptible check cv. Grazia were included in each block to check the level and distribution of the infection in the experimental fields. The RILs were evaluated in 2.4 m^2 plots (eight 2.0 m-long, solid-seeded rows) sowed at a density of 400 viable seeds m^{-1}. Meridiano, Claudio and Grazia were included with six plots per block distributed at regular intervals. All field trials were sown in early November, supplied with nitrogen (as urea) at the beginning of March (tillering phase), managed following the agronomic practices commonly adopted in the area, and harvested at the end of June.

Phenotypic evaluation of SBCMV response and related morpho-physiological traits

For the RIL population, in each season, symptom severity (SS) was visually scored on four dates (see below) during the plant growth stages showing the maximum disease symptom intensity, i.e., from mid-end of tillering (Z25 in the Zadoks scale, Zadoks et al. 1974) to first (Z31) and second (Z32) node appearance, corresponding to a period from the end of February to April. SS was recorded using a 0–4 scale (Vallega and Rubies Autonell 1985) where: 0–1.0 = no or slight symptoms; 1.1–2.0 = mild mottling and stunting; 2.1–3.0 = mottling and stunting and 3.1–4.0 = severe mottling and stunting, with virus-killed plants. Virus concentration was determined on homogenized extracts from leaves collected on two dates per year (see below), in coincidence with SS evaluation, using DAS (double antibody sandwich) ELISA according to Clark and Adams (1977) modified as follows: leaf sap extract was diluted in 1:6 saline phosphate buffer (pH 7.2) containing 0.05% Tween-20, 2% polyvinyl-pyrrolidone (MW 24,000), 0.2% powdered chicken albumin and 0.5 M urea. The extract was obtained from a bulk of the basal portion of the second and third youngest leaves of 15 randomly chosen plants per plot. The antiserum was prepared using SBCMV purified from infected plants of cv. Grazia grown in the field trial. Infection by *Wheat spindle streak mosaic virus* (WSSMV), a soil-borne pathogen also present in Italy (Rubies-Autonell and Vallega 1987), was excluded by ELISA tests.

On the same dates when the SS and ELISA traits were assessed, the following physiological traits were recorded: (i) canopy reflectance (related to the total plot biomass; Marti et al. 2007), measured as normalized difference vegetation index (NDVI) using a portable spectroradiometer (Green seeker Hand Held TM optical sensor unit, model 505, Ntech Industries, CA, USA) and (ii) leaf greenness (related to the leaf chlorophyll content), evaluated on 30 representative leaves per plot by means of a SPAD-502 handheld chlorophyll meter (Minolta).

The time course of the visual and physiological assessments was as follows:

	2007			
	February 16th (1-07)	February 21th (2-07)	March 12th (3-07)	April 2nd (4-07)
SS	×	×	×	×
ELISA		×		×
NDVI		×		×
SPAD		×		×
	2008			
	March 3rd (1-08)	March 11th (2-08)	March 25th (3-08)	April 10th (4-08)
SS	×	×	×	×
ELISA		×		×
NDVI	×	×	×	×
SPAD		×	×	×

For all traits, each assessment is hereafter indicated with an acronym including the trait itself, a progressive number corresponding to the scoring/sampling date order and the year (e.g., ELISA 2-07 = ELISA evaluated on 21 February 2007).

Grain yield (GY), thousand kernel weight (TKW) and seed weight per volume (test weight, TW) of the RILs were also measured. Fertility was assessed as the number of kernels per square meter (Kpsm) and was obtained indirectly using GY and TKW values.

The AM panel was evaluated for SBCMV-response traits (SS and ELISA) only, following the methodology described above for the M × C population. SS was scored on two dates both in 2003 (13 March and 13 April) and 2004 (30 March and 22 April). ELISA values were determined on leaf samples collected on the same dates.

Molecular analysis and linkage map

The 181 M × C RILs were genotyped with 212 simple sequence repeat (SSRs), 322 DArT® markers as well as with the allele-specific PCR assays tagging the *VRN-A1* locus. The SSR primer pairs, which are catalogued in the

GrainGenes database (http://wheat.pw.usda.gov), were from the following sets: BARC (*Xbarc* marker loci), CFA (*Xcfa*), CFD (*Xcfd*), CNL (*Xcnl*), KSUM (*Xksum*), GPW (*Xgpw*), WMC (*Xwmc*) and WMS (*Xgwm*). A small subset of private WMS primers, provided by Martin Ganal (TraitGenetics, Gatersleben, Germany), was also used. A unique thermocycling protocol was adopted for all SSRs: 94°C, 3 min; 20 cycles of touchdown PCR including 94°C, 45 s; 61/51°C, 45 s (−0.5°C/s); 72°C, 60 s, followed by 23 cycles including 94°C, 45 s; 51°C, 45 s; 72°C, 60 s, with a final extension at 72°C for 10 min. SSR profiles of the RILs were obtained using either 3% agarose gel electrophoresis (in case of differences in molecular weight between the two parental alleles ≥10 bp) or the automated LI-COR 4200 IR^2 System (LiCor, Lincoln, NE, USA) with forward primers labeled with IR700/IR800 fluorochromes. The DArT® markers were obtained at Triticarte (Yarralumla, Canberra, AU), using the Durum *Pst*I (*Taq*I) v 2.0 Array.

Construction of the linkage map

A linkage map including 33 linkage groups, for a total length of 2,140 cM was assembled using JoinMap v. 4.0 (Van Ooijen 2006).

Marker grouping was performed using the independence LOD method with LOD threshold range from 2.0 to 10.0. Robust initial linkage groups (LG) were obtained by selecting the groups with stable marker composition in the LOD range from 6.0 up to 10.0. The corresponding maps were obtained based on the maximum likelihood mapping algorithm. Mapping was based on repeated rounds of (i) *simulated annealing* Monte Carlo map order optimization search, followed by (ii) *Gibbs sampling* (Monte Carlo Expectation Maximization algorithm), which was used to obtain the multipoint recombination frequency estimates. For map building, the *number of map optimization rounds* was set equal to five. The LG maps were gradually built by taking *spatial samples* of loci using the five default recombination frequency thresholds (0.10, 0.05, 0.03, 0.02, 0.01). In the *simulated annealing* phase, a chain of 5,000 trial and error steps was used; the stop was set after reaching the number of 1,000 chains without further improvement; other parameters were set as default. In the *Gibbs sampling* phase, the length of the burn-in chain was set equal to 5,000 iterations and the chain length per Monte Carlo EM cycle was set to 1,000 iterations; other parameters were set as default.

For each linkage group, the marker order was checked using the *plausible position* calculation option (simulated annealing method) set to 1,000 random replacement samples of markers. Markers reported to cause unstable order were discarded. The assignment of linkage groups to wheat chrs. was carried out by checking for SSR loci common to other wheat maps as reported in the GrainGenes database. Centromere positions were inferred from previous mapping results of centromeric SSRs (Röder et al. 1998; Somers et al. 2004).

The *T. durum* elite germplasm collection was profiled with a total of 81 SSRs, 70 of which were evenly distributed over the 14 chrs. of the A and B genomes, while the remaining 11 were selected for mapping in the distal region of chr. 2BS found to harbor the major SBCMV-resistance QTL in the M × C population. The evenly distributed SSRs were used to estimate the population structure and the familial relationships among and within the groups of accessions (for further details, see Maccaferri et al. 2005), while the 11 SSRs localized in the QTL region were subjected to marker–trait association tests as described below. The relative map position and the genetic distances among markers have been assumed as in the *T. aestivum* consensus map (Ta-SSR-2004, Somers et al. 2004).

Statistical analysis of the phenotypic traits

Frequency distributions of the phenotypic data were inspected to assess the consistency of data through scoring/sampling dates and years and to investigate the complexity of the genetic control of the traits. The Box-Cox normality plot analysis was carried out in MINITAB statistical software v. 15.0 (Minitab Ltd, Coventry, England) for all the phenotypic data set considered. Based on the trait distributions and the Box-Cox analysis results, it was decided to use the square root transformation $\left(Y = \sqrt{X + 0.5}\right)$ for the SS data only before proceeding with the statistical analyses. ANOVA was carried out separately for each trait, scoring/sampling date and year; subsequently, combined ANOVA over scoring/sampling dates per year and over the 2 years were conducted for all traits. Pearson's correlation coefficients and the corresponding significance levels between SBCMV-response traits (SS and ELISA) and all the other traits (NDVI, SPAD, GY, TKW, KPSM and TW) were calculated in MINITAB. The heritability (h^2) values were calculated on a mean basis across three replications according to the following:

$$h^2 = \sigma^2_G / \left(\sigma^2_G + \sigma^2_E / r\right)$$

where r is the number of reps, $\sigma^2_G = \left(MS_{genotypes} - MS_{error}\right)/r$ and $\sigma^2_E = MS_{error}$ with MS indicating the mean square values.

Heritability should be considered as being ''narrow sense'' because the genetic variance included only the additive component and, possibly, the additive × additive epistatic interaction.

QTL analysis

A subset of 295 markers with minimum intermarker distance equal to three cM and low incidence of missing data was chosen to conduct the QTL analysis (average intermarker distance of ca. 7 cM) in the RIL population. Single-marker analysis using linear regression and composite interval mapping (CIM) were carried out in Windows QTL Cartographer v. 2.5 (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm). The following parameters in 'Model 6 standard analysis' were used: (i) walk speed of 2 cM step, (ii) manual selection of the control markers (cofactors) based on the output of a 'forward & backward' regression analysis conducted with a 0.05 probability level for in and out, and (iii) blocked window size of 10 cM to exclude closely linked control markers at the testing site. The LOD threshold for declaring the presence of a significant QTL for each trait–environment combination was defined by 500 permutations at P 0.05 (Churchill and Doerge 1994): the LOD threshold scores (= LR × 0.217) for all the considered traits were quite close to 3 (data not reported), thus a common LOD threshold = 3 was used.

For each trait, the additive effect was computed as half of the phenotypic difference between the mean values of the RIL groups homozygous for the Claudio and the Meridiano alleles at the QTL peak position.

A subset of 86 RILs with the unbroken Claudio (susceptible) molecular haplotype at the major SBCMV-resistance QTL was used for an additional analysis carried out to better investigate for the presence of QTLs with minor effects. In this case, QTLs with LOD score peaks higher than 3.0 were considered as 'high-confidence' QTLs; additionally, QTLs with LOD score peaks between 2 and 3 were also reported and considered as 'low-confidence' QTLs, provided that the same chr. region harbored overlapping QTLs (QTL peaks within 20 cM) in two or more scoring/sampling dates and/or years.

Association mapping

An AM approach based on a durum panel was used to validate and further explore the frequency and the phenotypic effects of the SSR haplotype associated with the resistant allele at the major SBCMV-resistance QTL identified in the RIL population. The genetic structure of the panel has been assumed as reported in Maccaferri et al. (2005): the optimum number of subgroups describing the genetic structure of the collection was equal to six ($Q = 6$). The membership coefficients to the six subgroups (estimated as percentage of the genome of each accession belonging to each subgroup) were used to qualitatively assign each accession to the subgroup with the highest membership coefficient value. The 6 × 111 membership coefficient Q matrix was used to investigate the relationships between the population structure and the SBCMV responses of the accessions by means of multiple regression analysis (MINITAB software); the same matrix was used as covariate in the structured marker–trait association tests.

In order to account for further subtle familial relationships among accessions, a 111 × 111 co-ancestry (kinship) K matrix was obtained based on the simple matching genetic distances among all the pairwises of accessions computed with NTSYS-pc Software Package V 2.0 (Rohlf 1997) using the set of 70 evenly distributed SSR loci (Maccaferri et al. 2007).

The LD estimates (D' and P values) for each pair of SSRs mapping in the major SBCMV-QTL region were calculated as in Weir (1996) and Farnir et al. (2000) according to the equation for markers with multiple alleles. The LD P values were estimated using 10,000 permutations.

Association of the allelic variants at the 11 SSR loci targeting the QTL and the SBCMV-response traits has been carried out using the general linear model (GLM) accounting for the genetic structure of the collection (Q matrix used as covariate) and the mixed linear model (MLM) including the Q and the K matrices. Only alleles with a frequency higher than 10% in the collection (hereafter indicated as common alleles) were considered in the association test. LD estimates and marker–phenotype association tests were carried out in TASSEL 2.0.1 software (Bradbury et al. 2007). For the markers significantly associated to the phenotype, the R^2 values and the least square means of the allelic variants were calculated.

Results

The SBCMV-infection level detected in all the field experiments herein considered was quite uniform, as indicated by the limited variability for SS and ELISA values across the replicated plots of the highly susceptible check cv. Grazia (data not reported). Moreover, as shown by the high SBCMV-response values of Grazia (Tables 1, 2), the infection levels were high and adequate for a thorough evaluation of the disease response. Mosaic symptoms persisted in the leaves of Grazia as well as in those of other susceptible genotypes beyond heading time in all experiments.

SBCMV response and related traits in the Meridiano × Claudio RIL population

ANOVA evidenced significant (P 0.01) differences for all traits both among RILs and between the two parents (data not shown). The combined analysis of SS scores and

Table 1 Mean symptom severity (SS), virus concentration (ELISA absorbance values) and grain yield (GY) for Meridiano and Claudio (parents of the recombinant inbred population), for Grazia (susceptible check) and for the 181 $F_{7:8}$ M × C recombinant inbred lines (RILs) evaluated in 2 years (2007 and 2008) of field trials

	SS				ELISA		GY	SS				ELISA		GY
	1-07[a] (Visual score)[b]	2-07	3-07	4-07	2-07 (Absorbance)[c]	4-07	07 (t ha^{-1})	1-08 (Visual score)	2-08	3-08	4-08	2-08 (Absorbance)	4-08	08 (t ha^{-1})
Meridiano	0.06	0.03	0.06	0.30	0.83	1.20	5.56	0.00	0.25	0.00	0.33	0.27	0.44	8.72
Claudio	1.93	2.13	2.53	2.70	1.41	1.91	1.87	0.43	2.28	1.75	2.18	1.86	1.60	6.06
Grazia	3.18	3.13	3.43	3.80	1.47	1.78	0.99	2.03	3.45	3.58	3.47	2.03	2.03	2.19
RILs														
Mean	1.28	1.39	1.22	1.61	1.15	1.40	3.41	0.56	1.68	1.29	1.44	1.10	1.16	6.64
Min	0.00	0.00	0.00	0.00	0.10	0.10	0.28	0.00	0.00	0.00	0.00	0.00	0.00	1.45
Max	3.25	3.38	3.13	4.00	1.80	2.00	6.99	3.00	4.00	3.90	3.90	2.11	2.07	10.96
CV	16.1	13.8	9.1	11.3	32.5	27.9	26.9	23.4	18.7	13.4	13.1	54.3	52.9	17.4
h^2 (%)	88.3	92.4	97.6	96.1	64.3	78.2	89.2	72.2	84.7	94.5	93.3	68.2	71.4	82.6

Range (minimum and maximum), coefficient of variation (CV) and heritability values (h^2) of the M × C RILs are also reported

[a] Scoring/sampling dates are indicated in "Materials and methods"

[b] Visual score from 0 (no symptoms) to 4 (severe mottling and stunting, with virus-killed plants)

[c] Absorbance: $A_{405\ nm}$

ELISA determinations in subsequent dates for each year showed significant differences among dates (P 0.01) in both years as well as a significant "RIL × scoring/sampling date" interaction (P 0.05). Combined ANOVA over the 2 years indicated that in most cases the first and second order interactions involving years were significant (P 0.05). Based on these results, each trait in each date and year has been analyzed and discussed separately.

The mean values of the two parents and of cv. Grazia together with the means and ranges of the RILs, the coefficient of variation (CV) and the heritability estimates (h^2) for all response traits (SS and ELISA) and GY are reported in Table 1. The overall infection level in 2007 was slightly higher than in 2008, with SS mean values across dates equal to 1.39 in 2007 and to 1.25 in 2008. In both seasons, the SBCMV response of the parental cvs. was consistent with previous long-term observations (Rubies et al. 2009). In particular, the resistance level of Meridiano was noticeably higher than that of Claudio, which consistently showed a susceptible response, even if at a level inferior to that of the highly susceptible check cv. Grazia. Considering all the scoring/sampling dates of the 2 years, SS values ranged from 0.06 to 0.33 for Meridiano and from 0.43 to 2.70 for Claudio and ELISA values ranged from 0.27 to 1.20 for Meridiano and from 1.41 to 1.91 for Claudio.

Notwithstanding the lower yield potential of Meridiano compared to that of Claudio under normal cultivation conditions (Desiderio et al. 2007; Giunta et al. 2007), in the SBCMV-infected field trials the GY of Meridiano was much higher (5.56 and 8.72 t ha^{-1} in 2007 and 2008, respectively) than that of Claudio (1.87 and 6.06 t ha^{-1} in 2007 and 2008, respectively). The lower GY of Claudio was mainly due to its lower number of kernels m^{-2}, while only slight differences as to TKW and TW were detected between the two parents (Supplemental Table 1). Moreover, Claudio showed NDVI and leaf greenness values (SPAD) consistently lower than those of Meridiano across all the scoring dates over the two seasons (Supplemental Table 2).

The RIL frequency distributions (Supplemental Fig. 1) for SS and ELISA were scarcely informative, except for evidencing substantial deviations from the normal distribution as well as the presence of transgressive segregation, both consistently observed across sampling dates and years. Although deviation from the normal distribution toward a bi-modal distribution was evident, the absence of clear bi-modality suggested that additional resistance factors were segregating in the population in addition to a major gene. Transgressive segregants for susceptibility were present for SS and ELISA while transgressive segregation for resistance was clearly detected for ELISA values only. RILs completely resistant to SBCMV were not recovered. Indeed, leaf samples from all the RILs assayed were found ELISA-positive in at least one of the four collection dates, and all except two RILs showed ELISA-positive results on at least two dates.

The h^2 values were medium to high for SS (from 72.2 to 97.6%) and slightly lower for ELISA values (from 64.3 to 78.2%); the latter trait, moreover, evidenced higher CVs (Table 1). In general, SS h^2 values increased (and CV decreased) moving toward the latest scoring dates (SS3 and SS4) of each year. A similar trend was observed for ELISA values. GY, a trait strongly affected by SBCMV infection,

Table 2 Mean values, range (minimum and maximum), coefficient of variation (CV) and heritability values (h^2) of the association panel (111 elite durum wheat accessions) for symptom severity (SS) and virus concentration (ELISA) over 2 years (2003 and 2004)

	Accessions		SS		ELISA		SS		ELISA	
	(no.)		1-03 (Visual score)[a]	2-03	1-03 (Absorbance, $A_{405\ nm}$)	2-03	1-04 (Visual score)	2-04	1-04 (Absorbance, $A_{405\ nm}$)	2-04
Grazia (check)			2.72	2.67	1.41	1.35	2.82	3.30	1.65	1.81
	111	Mean	1.09	1.09	0.69	0.54	1.44	1.64	0.70	0.87
		Min	0.00	0.00	0.03	0.02	0.00	0.00	0.00	0.02
		Max	3.09	3.32	1.70	1.71	3.60	3.55	1.81	1.83
		CV	32.6	26.1	17.2	30.5	30.3	21.2	15.8	11.4
		h^2	68.5	74.1	85.6	79.3	70.8	87.4	88.8	91.5
Subgroup 1[b]	9	Mean	0.84	1.21	1.05	0.93	1.28	2.03	0.99	1.26
Subgroup 2[c]	16	Mean	1.06	0.71	0.42	0.27	1.21	1.42	0.39	0.48
Subgroup 3[d]	19	Mean	1.72	1.80	1.10	0.86	2.29	2.31	1.06	1.30
Subgroup 4[e]	20	Mean	1.37	1.67	0.96	0.83	1.96	2.40	1.05	1.27
Subgroup 5[f]	35	Mean	0.82	0.55	0.33	0.17	0.83	0.80	0.31	0.42
Subgroup 6[g]	12	Mean	0.67	0.97	0.75	0.68	1.43	1.62	0.88	0.94
ANOVA										
P			0.004	0.001	0.001	0.001	0.001	0.001	0.001	0.001
LSD			0.51	0.51	0.34	0.33	0.65	0.59	0.37	0.39
Multiple regression										
R^2 (%)			19.50	32.40	30.40	31.40	32.00	43.40	28.90	32.80

Mean values and results of the analysis of variance (*P* and least significant difference, LSD) for the SBCMV responses of the six subgroups present in the association panel are reported. Multiple regression R^2 value of the SBCMV-response traits versus the accession subpopulation membership coefficients is also reported

[a] Visual score from 0 (no symptoms) to 4 (severe mottling and stunting, with virus-killed plants)

[b] Italian and Mediterranean native germplasm

[c] Elite accessions from CIMMYT germplasm (1980)

[d] Elite accessions from Italy (founder Valnova)

[e] Elite accessions from the CIMMYT-ICARDA germplasm (1970–1980; founders Jabato and Creso)

[f] Elite accessions of North American and French origin

[g] Elite accessions from Austria

was the second-ranking trait as to h^2 values (89.2 and 82.6% in 2007 and 2008, respectively; Table 1 and Supplemental Tables 1 and 2).

Correlations between all the measured traits were highly significant in all cases (data not shown). SS scores recorded on different dates were correlated with each other across sampling dates and years (r values ranging from 0.70 to 0.96). The correlation coefficient between the two consecutive ELISA determinations was equal to 0.75 and 0.70 in 2007 and 2008, respectively. Relatively high correlation coefficients were observed between SS and ELISA (from 0.63 to 0.79) as well as between the disease response traits and GY (r always higher than 0.80 and 0.60 for SS and ELISA, respectively). RILs showing higher SBCMV susceptibility were characterized by lower total plot biomass and leaf chlorophyll content, as evidenced by the negative r values (from −0.60 to −0.80) between the disease response traits (SS and ELISA) and NDVI or SPAD.

SBCMV response in the germplasm collection

The disease pressure in the germplasm assays was high, as indicated by the SS values (2.7 and 3.3 in 2003 and 2004, respectively) recorded on the check cv. Grazia (Table 2). Highly significant differences were detected among accessions for SS and ELISA in each scoring/sampling date and year (data not reported). The interactions between accessions and dates or years were also significant (data not reported).

As observed in the M × C population, the frequency distributions of the germplasm phenotypic data for SS and ELISA suggested a complex inheritance of the SBCMV-response traits (Supplemental Fig. 1). In general, medium to high h^2 values (always higher than 60% and often close to 80%) were observed for both SS and ELISA values (Table 2), thus indicating a prevalent contribution of the genotypic component to the phenotypic variability.

Table 2 reports also the mean phenotypic values of the six germplasm subgroups as identified in the population structure analysis, the results of ANOVA among such subgroups and the R^2 values of the multiple regression of phenotypes on germplasm structure estimates (i.e., membership coefficients). Overall, population structure strongly influenced the SBCMV response, as shown by the high multiple regression R^2 values of population structure estimates vs. SS and ELISA; such values, in fact, ranged from 19.5 to 43.4% for SS scores and from 28.9 to 32.8% for ELISA. The differences among subgroups were significant (P 0.01) for both SS and ELISA. Elite Italian and CIMMYT-ICARDA accessions (subgroups 3 and 4, respectively) showed the highest mean values for SS and ELISA across dates and years, whereas the accessions of North American and Austrian origin (subgroups 5 and 6, respectively) were characterized by the lowest mean values. However, susceptible and resistant genotypes were represented within all subgroups. This observation indicates that fixation of alleles at the causative SBCMV-response loci did not take place within each sugroup, thus reducing the occurrence of Type II errors (also known as false negatives), i.e., the incorrect acceptance of the null hypothesis (stating that the considered marker is not associated to a causative SBCMV-response locus) due to strong population structure confounding effect.

Genetic control of SBCMV response and related traits in the Meridiano × Claudio RIL population

QSbm.ubo-2BS, *a major QTL for SBCMV resistance*

Simple marker regression and CIM analyses conducted on the complete data set from the 181 RILs showed the presence of a major QTL (*QSbm.ubo-2BS*) for SBCMV response consistent across sampling dates and years. *QSbm.ubo-2BS* was located in the distal region of chr. arm 2BS and, based on the CIM analysis of the SS and ELISA data, it was more precisely positioned in a reasonably short interval (LOD-2 confidence interval = 12 cM) between positions 6 and 18 cM of the linkage group (see Fig. 1 for the LOD traces from the CIM scans and Table 3 for the statistical features of the QTL). The nearest marker was wPt-2106, a DArT marker located 3.4 cM proximal to *Xbarc35*. The effect of *QSbm.ubo-2BS* on SBCMV response was rather strong and the favorable allele conferring the resistant response was inherited from Meridiano. In both years, the highest LOD and R^2 values for SS were observed at scoring dates 3 and 4 (late March-beginning of April), i.e., during the period characterized by the highest disease symptom scores. The maximum LOD values for SS were obtained for SS3 in 2007 and in SS4 in 2008 (LOD values equal to 103.0 and 92.3, respectively)

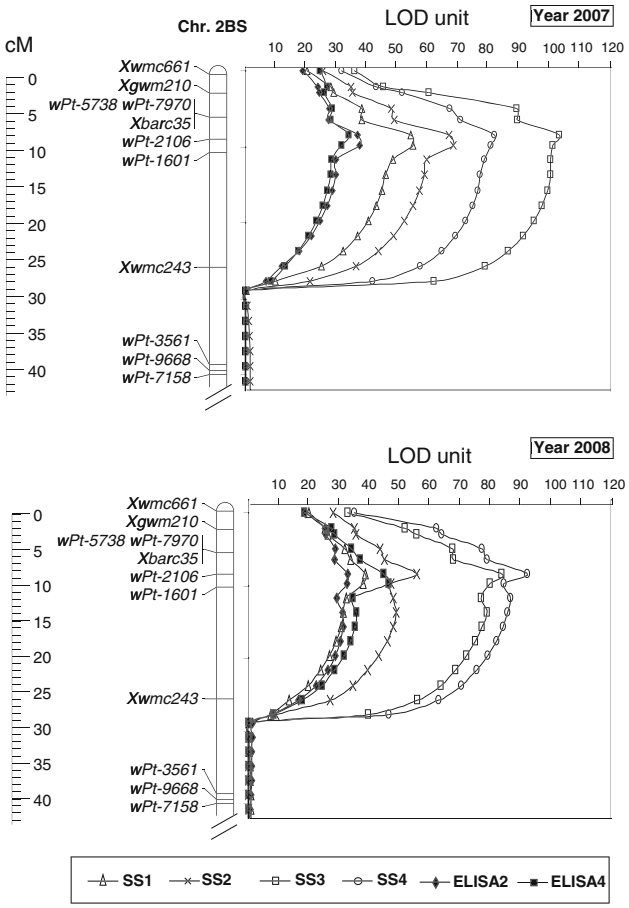

Fig. 1 LOD score plots at the major QTL (*QSbm.ubo-2BS*) identified for symptom severity (SS) score and virus concentration (ELISA) in the distal region of chromosome 2BS using the Meridiano × Claudio recombinant inbred population. Data were recorded in four scoring/sampling dates in 2007 and 2008

and the corresponding R^2 values were equal to 91.6 and 85.3%. As compared to SS, virus concentration data showed lower LOD peak and R^2 values (from 32.9 to 46.6 for LOD values and from 51.6 to 68.0% for R^2 values.

QSbm.ubo-2BS significantly affected also GY in both years (Table 3), with R^2 values equal to 69.7 and 64.6% in 2007 and 2008, respectively. On average, the 87 RILs homozygous at the marker nearest to the QTL peak (wPt-2106) for the Meridiano resistance allele out-yielded the 83 RILs homozygous for the Claudio allele.

A favorable effect of the resistance allele was also recorded for all SPAD measurements (except SPAD2-2008), NDVI, TW, and the yield components. In particular, the differences between the two RIL groups were equal to 1.82 and 2.66 kg hl^{-1} for TW, 7,496 and 7,326 kernels m^{-2} for KPSM, and 5.00 and 2.48 g for TKW in 2007 and 2008, respectively (Supplemental Table 3).

The results of the single-marker QTL analysis performed for SS4 and ELISA4 in both years using the markers mapping in the distal region (from 0 to 60 cM) of

Table 3 Features of *QSbm.ubo-2BS,* the major locus for SBCMV response, identified in the most distal region of chromosome 2BS based on the composite interval mapping (CIM) analysis of 181 recombinant inbred lines (RILs) obtained from the cross Meridiano × Claudio

Trait	QTL position (cM)[a]	Most associated marker	LOD peak (LOD unit)	R^2 (%)	Additive effect[b]
SS1-07[c]	6-10-12	wPt-2106	55.2	69.6	+0.91
SS2-07	12-14-18	wPt-1601	58.9	79.0	+1.06
SS3-07	6-8-18	wPt-2106	103.0	91.6	+1.22
SS4-07	6-8-10	wPt-2106	81.9	81.3	+1.39
ELISA2-07[d]	6-10-12	wPt-2106	37.6	55.8	+0.33
ELISA4-07	6-8-16	wPt-2106	34.0	55.2	+0.40
GY-07[e]	6-8-10	wPt-2106	61.0	69.7	−1.73
SS1-08	6-8-12	wPt-2106	38.8	59.4	+0.50
SS2-08	6-8-8	wPt-2106	55.9	67.5	+1.16
SS3-08	6-8-10	wPt-2106	83.9	84.6	+1.18
SS4-08	6-8-10	wPt-2106	92.3	85.3	+1.22
ELISA2-08	6-8-12	wPt-2106	32.9	51.6	+0.54
ELISA4-08	8-10-12	wPt-2106	46.6	68.0	+0.67
GY-08[e]	6-8-12	wPt-2106	49.3	64.6	−1.60

The QTL analysis results for symptom severity (SS) and virus concentration (ELISA) are reported separately for each scoring/sampling date (from 1 to 4) and year (2007 and 2008)

[a] The central value indicates the QTL peak position; flanking values indicate the LOD-2 confidence interval

[b] Calculated as half of the difference between the mean value of the RILs homozygous for the Claudio allele and the mean value of the RILs homozygous for the Meridiano allele. For SS and ELISA, the favorable QTL allele (conferring resistance to SBCMV) was contributed by Meridiano. Allelic effects positive in sign indicate that the allele increasing the trait originates from Claudio (susceptible parent)

[c] SS as visual score from 0 (no symptoms) to 4 (severe mottling and stunting, with virus-killed plants). Scoring/sampling dates are indicated in "Materials and methods"

[d] ELISA as absorbance units at 405 nm

[e] GY as t ha^{-1}

chr. arm 2BS have been reported in Supplemental Table 4. All the markers found to be associated (P 10^{-4}) to SBCMV response were in the 0–12 cM chr. interval (from *Xwmc661* to wPt-1601), i.e., in coincidence with *QSbm.ubo-2BS,* and with *Xbarc35* (cosegregating with wPt-5738 and wPt-7970), wPt-2106 and wPt-1601 being the most associated ones.

Based on the 2-year phenotypic SS data set (four subsequent scoring dates/year), most of the RILs were classified as either resistant (R: SS $\leq$0.5 in at least five scoring dates and overall mean <0.5) or susceptible (S: SS $\geq$2.0 in at least five dates and overall mean >2.0); a limited number of RILs were classified as medium susceptible (MS) or medium resistant (MR). It was thus possible to easily compare the correspondence between the molecular information at the *QSbm.ubo-2BS* region and the categorized phenotypic SBCMV response. Among the 77 RILs showing a long-range haplotype identical to that of the susceptible parent Claudio in the *QSbm.ubo-2BS* region (*Xwmc661-Xwmc243*), 72 were classified as 'S' and five as 'MS'. Analogously, among the 73 RILs with the haplotype identical to that of the resistant parent Meridiano, 69 were classified as 'R' and four as 'MR'.

Figure 2 reports the graphical genotypes of the 31 RILs, which showed recombination events in the *Xwmc661-Xwmc243* interval; these RILs were classified as 'R' or 'S', except for four RILs whose phenotypes were intermediate (probably due to residual heterogeneity within RIL). In particular, the RILs with the Meridiano allele at wPt-2106 and wPt-1601 were resistant, while those with the Claudio allele were susceptible. This finding together with the categorized phenotype of the six RILs recombinant in the 2 cM interval between wPt-2106 and wPt-1601 suggested that *QSbm.ubo-2BS* is most probably located in this chr. region. Moreover, the graphical haplotypes of the RILs clearly indicated that the terminal chr. region distal to wPt-2106 most probably did not harbor *QSbm.ubo-2BS* (presence of seven resistant lines with the susceptible allele at *Xbarc35*).

Minor QTLs for SBCMV response

To recognize and estimate the effects of minor QTLs for SBCMV response, selective single-marker and CIM analyses were carried out on a subset of 85 RILs with the molecular haplotype at the *Xbarc35*–wPt2106–wPt1601 interval identical to Claudio (susceptible).

	Xwmc661	*Xgwm210*	wPt-5738	wPt-7970	*Xbarc35*	wPt-2106	wPt-1601	*Xwmc243*	SBCMV-response[b]
Map position[a]	0	2.8	6.4	6.4	6.4	9.7	11.7	28.9 cM	
MxC RIL[c]									
MC006	C	C	C	C	C	M	M	M	R
MC068	C	C	C	C	C	M	M	M	R
MC053	C	C	C	C	C	M	M	M	R
MC180	C	C	C	C	C	M	M	-	R
MC202	C	C	C	C	C	M	M	-	R
MC082	C	C	M	M	M	M	M	M	R
MC090	C	C	M	M	M	M	M	M	R
MC149	C	M	M	M	M	M	M	C	R
MC154	C	M	M	M	M	M	M	M	R
MC156	C	M	M	M	M	M	M	C	R
MC158	C	M	M	M	M	M	M	M	R
MC160	C	M	M	M	M	M	M	C	R
MC084	-	M	C	C	C	M	M	M	R
MC023	C	M	-	C	C	M	M	M	R
MC079	M	M	M	M	M	C	C	C	S
MC095	-	M	C	C	C	C	C	C	S
MC339	M	M	C	C	C	C	C	C	S
MC267	M	M	C	C	C	C	C	C	S
MC193	-	M	-	M	C	C	C	M	S
MC310	C	M	-	-	C	C	C	C	S
MC096	M	C	C	C	C	C	C	C	S
MC012	M	M	M	M	M	M	C	C	R
MC099	M	M	M	M	M	M	C	C	R
MC347	M	M	M	M	M	C	M	M	R
MC008	-	M	M	-	M	C	M	M	R
MC223	C	C	C	C	C	C	M	-	S
MC104	C	C	C	C	C	C	M	M	S
MC005	-	M	C	C	C	C	-	-	MR
MC270	-	M	C	C	C	-	-	C	MR
MC145	-	M	C	C	C	C	-	C	MR
MC151	M	C	C	C	C	C	-	M	MS

[a] Marker order and map distances are estimated based on the Meridiano × Claudio intra-specific RIL durum wheat mapping population (181 lines).

[b] Overall phenotypic response of the RILs to SBCMV infection, based on a phenotypic severity symptom score data set obtained from two field experiments (2007 and 2008). The RILs were classified as resistant (R), medium-resistant (MR), medium-susceptible (MS), or susceptible (S); for details see the results section).

[c] Graphical genotypes of the RILs at four SSRs and four DArT markers: the alleles inherited from the parents Meridiano and Claudio are reported with the capital letters M and C, respectively, while the undefined and/or heterogeneous RIL allelic compositions are reported with the "-" symbol.

Fig. 2 SSR and DArT marker-based graphical genotypes of 31 Meridiano × Claudio recombinant inbred lines (RILs) showing recombinations in the distal region (from *Xwmc661* to *Xwmc243*) of chromosome 2BS harboring *QSbm.ubo-2BS*. The categorized SBCMV response, based on 2 year severity symptom scores, is reported for each RIL side by side with the corresponding graphical genotype. The RILs have been ordered based on their genotypes in the interval that most likely includes the QTL, i.e., between wPt-2106 and wPt-1601

The locations of the minor QTLs identified with CIM are depicted in Fig. 3 and the detailed results for SS, ELISA values and GY are reported in Supplemental Table 5. A total of 11 minor QTLs (including also the low-confidence ones), which affected SBCMV response (either as SS or ELISA) in one or more scoring/sampling dates, were identified. The LOD value at the QTL peaks ranged from 2.0 to 8.1 and the R^2 values from 7.0 to 30.1%. It should be noted that QTLs with LOD values between 2 and 3 were reported and considered as low-confidence QTLs only in case the QTLs were observed for at least two SBCMV-response trait/date combinations.

Most of the 11 minor QTLs were consistently detected across consecutive scoring/sampling dates and/or years and four of them concomitantly affected also GY. Among these minor QTLs, *QSbm.ubo-5A*, located near *VRN-A1*, evidenced the highest LOD and R^2 values, with significant effects on SBCMV response consistently observed in 2007. This QTL showed analogous effects in the 2008 season even if it did not reach the significance treshold. It should be noted that the susceptible parent Claudio contributed both to the favorable *QSbm.ubo-5A* resistance allele as well as the winter allele (*vrn-A1c*).

Three minor QTLs evidenced significant effects for SBCMV-response traits in both years: *QSbm.ubo-1A* (SS1-07, SS2-07 and ELISA2-08), *QSbm.ubo-2A* (SS3-07 and SS2-08) and *QSbm.ubo-7B* (SS1-07, SS2-07, ELISA2-07, SS3-08 and SS4-08). Conversely, other minor QTLs were either year or trait specific.

Claudio carried the favorable (low susceptibility) alleles at 4 out of the 11 minor QTLs; the sign of the additive effects was always consistent across scoring/sampling dates and/or years.

Results of the CIM and single-marker regression analyses for all the considered traits are reported in Supplementary Fig. 2. It can be noted that many of the minor QTLs detected with CIM for SBCMV response showed concomitant effects on NDVI, SPAD, GY, TKW, KPSM and TW. In particular, eight QTLs significantly influenced GY or its components, and most of the QTLs significant for SS only showed concomitant associations to NDVI and/or SPAD. In addition to the chr. regions detected with CIM, single-marker regression evidenced some other regions showing significant (P 0.05) effects for SS and/or ELISA traits. Since in several cases the significant effects were consistent across scoring/sampling dates and years, such chr. regions may harbor further low-confidence QTLs.

Inheritance of *QSbm.ubo-2BS* in the durum wheat germplasm panel: association mapping results

QSbm.ubo-2BS features were further investigated by means of a germplasm collection of 111 durum accessions already characterized for SBCMV response (Ratti et al. 2006) and genotyped with 11 polymorphic SSRs evenly spaced on a 30-cM interval including the QTL region. It is interesting to observe that the Meridiano allele (hereafter A_{Mer}) was one of the common alleles (present with a frequency >10%) across all the SSR markers mapped in the QTL region; in particular the A_{Mer} counts ranged from a minimum of 49 accessions for *Xbarc35* to a maximum of 89 accessions for *Xgwm614*.

Genetic resolution in the germplasm collection at the QSbm.ubo-2BS *region*

The local LD decay pattern present in the target region was investigated and the pairwise P and D' LD values among the 11 SSR markers are reported in Fig. 4 using the marker

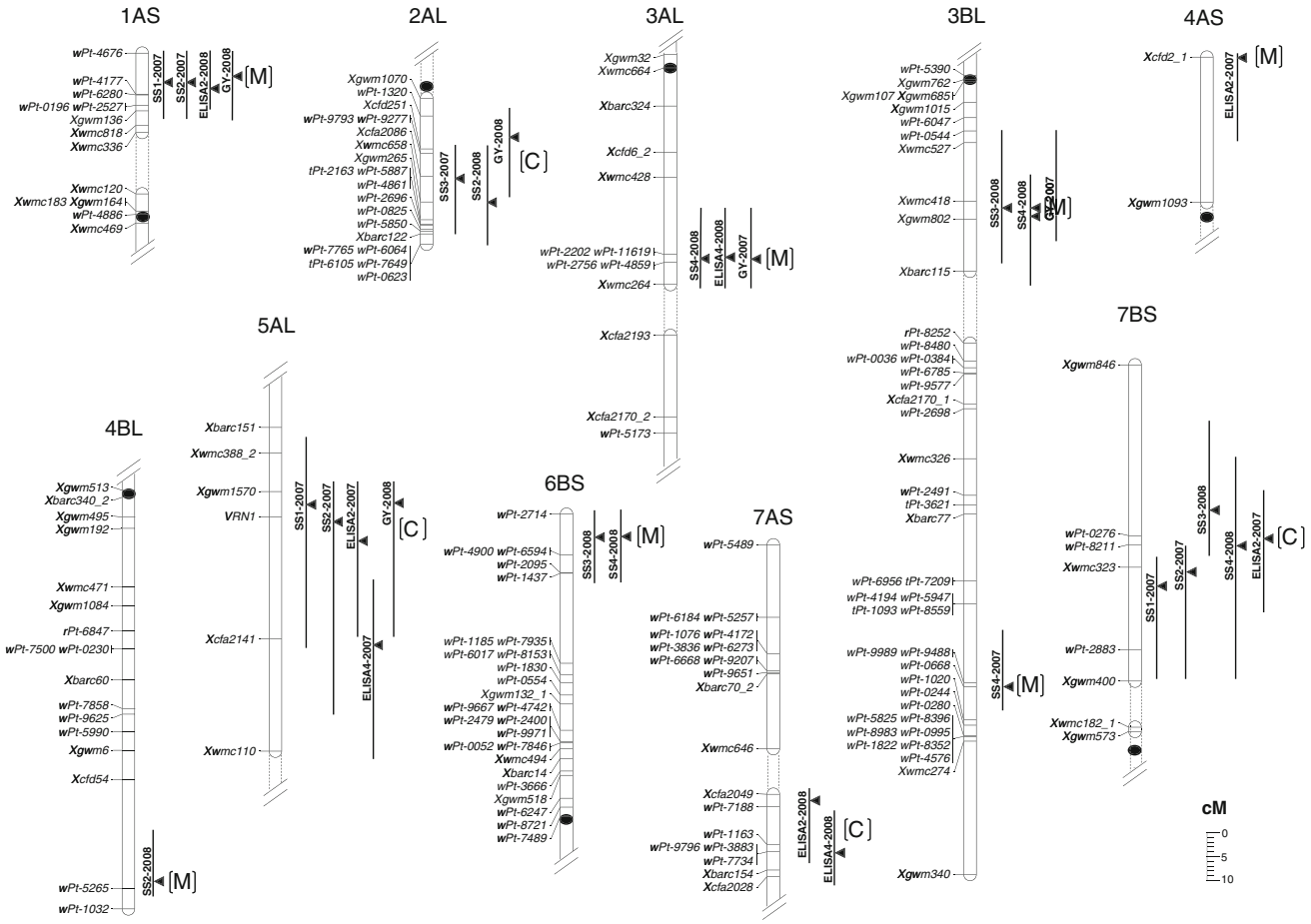

Fig. 3 Linkage groups harboring the minor QTLs for SBCMV-response traits identified in the Meridiano × Claudio RIL population. The minor QTLs were identified by composite interval mapping analysis on a subset of 89 RILs with the Claudio (susceptible) extended haplotype at *QSbm.ubo-2BS*. *Vertical bars* on the right side of the linkage groups indicate the QTL confidence interval (LOD-2). *Black filled triangles* point to the QTL peak position

order and relative distances as from the *T. aestivum* Ta-SSR-2004-2B consensus map (Somers et al. 2004). The LD among markers showed an irregular pattern across the region with rapid LD decay within a 5-cM range observed overall. More specifically, in the most distal portion, three markers (*Xbarc35*, *Xwmc661* and *Xgwm210*) located within 2.1 cM displayed high LD as to each other and were considered as a linkage block where the genetic variation present in the germplasm collection was represented by four common haplotypes only; one of them (the Meridiano haplotype) was present at high frequency (49 accessions), while the other three haplotypes showed a frequency ranging from 13 to 18 accessions. At the adjacent markers, *Xwmc489* and *Xwmc382*, LD decay and haplotype rearrangements were observed. Sliding along the linkage group, the presence of LD was observed between the triplet *Xbarc35-Xwmc661-Xgwm210* and the pair *Xgwm614-Xbarc124* (which were mapped ca. 10 cM away). Unexpectedly, the triplet of distal markers also showed strong LD with *Xwmc243*, a marker located ca. 30 cM proximal.

Marker–SBCMV response association

For each SSR, the marker–phenotype association test was conducted by contrasting the A_{Mer}, known to be associated with SBCMV resistance based on the biparental QTL study, against the pool of all the other common alleles (one or two alleles, depending on the marker). As from the results of the association tests (P values plotted in Fig. 5, detailed results of P and R^2 values reported in Supplemental Table 6), the distal region of the linkage group tagged by *Xbarc35-Xwmc661-Xgwm210* showed significant (P from 0.05 to 0.001) associations to SBCMV response, with *Xbarc35* and *Xwmc661* being the markers most consistently associated across all the SS and ELISA assessments carried out in the 2 years (eight in total). Consistently highly significant associations were observed only for the above group of distal markers and, unexpectedly, for the proximal marker *Xwmc243*, where the A_{Mer} allele was again strongly associated to the SBCMV response.

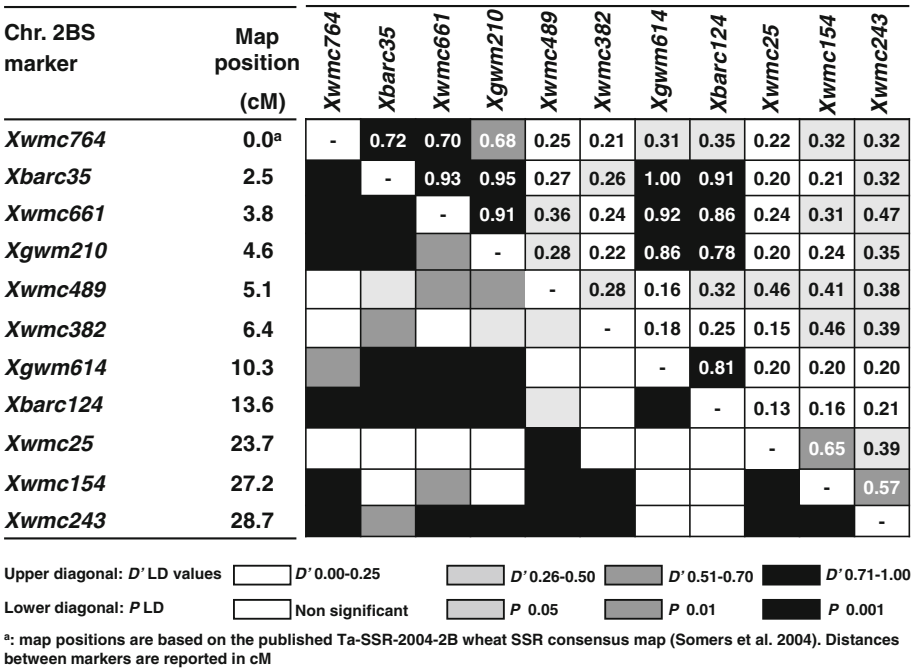

Fig. 4 Linkage disequilibrium (LD) estimates among SSR markers mapping in the chromosome 2BS distal region harboring *QSbm.ubo-2BS* in the elite germplasm collection of 111 elite accessions. LD D' and probability estimates (P) have been considered and reported above and below the *diagonal line*, respectively

At *Xbarc35* and *Xwmc661*, the percentage of phenotypic variation accounted for ranged between 10.0 (*Xbarc35* at SS1-03) up to 23.7% (*Xwmc661* for ELISA2-04). At *Xwmc243*, R^2 values ranged between 8.0 (SS1-04) and 26.7% (ELISA2-04). Generally, the R^2 values of the associations were higher for the SBCMV response recorded in 2004 as compared to 2003. For each significant marker–trait association, the least square mean allelic value of A_{Mer} was lower than that of the pool of the other alleles, i.e., on average the accessions with the Meridiano allele were more resistant than the others (see Supplemental Table 7).

Supplemental Table 8 reports the detailed molecular genotype, the molecular weight (base pairs) of the SSR alleles and the phenotypic values recorded for each accession. At *Xbarc35*, *Xwmc661* and *Xgwm210*, the Meridiano alleles occurred most frequently as a single unbroken Meridiano haplotype. In fact, out of 54 accessions with at least one allele identical in state to Meridiano at any of the three relevant markers, 49 were characterized by the unbroken Meridiano haplotype spanning all the three markers and 44 were classified as resistant or medium resistant based on both SS and ELISA data (for the SBCMV-response classification, the same criteria used for the RILs were adopted). Moreover, 24 additional accessions were classified as resistant to SBCMV while lacking the Meridiano haplotype at *Xbarc35-Xwmc661-Xgwm210*. Interestingly, among these 24 accessions, 13 displayed the Meridiano allele at the nearest marker (*Xwmc489*) in the proximal region and 21 at *Xwmc243*, which is located ca. 25 cM away from the QTL peak as evaluated in the RIL population. At *Xwmc243*, the Meridiano allele was present in 69 accessions, seven of which (10%) showed a clearly susceptible phenotype; further, 21 accessions were resistant or medium resistant although not carrying the Meridiano haplotype at *Xbarc35-Xwmc661-Xgwm210*.

Distribution of the SBCMV resistance-tagging haplotype at the QSbm.ubo-2BS *region in the germplasm collection*

Figure 6 shows the distribution of the Meridiano haplotype (based on the critical markers *Xbarc35-Xwmc661-Xgwm210*) in the 111 germplasm accessions togheter with the corresponding categorized phenotypes and the population structure membership's coefficients. The resistance-tagging haplotype at *Xbarc35-Xwmc661-Xgwm210* was observed in all the population subgroups except subgroup 1, which includes germplasm of Mediterranean origin only. Nonetheless, some of the subgroup 1 accessions evidenced a medium to high level of resistance (e.g., Appulo, Cappelli, etc.).

Resistant accessions with the Meridiano haplotype at the *QSbm.ubo-2BS* region were present at relatively high frequency in (i) subgroup 2, including the breeding lineage that originated from the CIMMYT Jori/Anhinga//Flamingo materials (widely diffused in the Mediterranean countries as Yavaros 79, Karim, Vitron, Duilio and Latino), (ii) subgroup 4 comprising CIMMYT/ICARDA and southwestern US cvs., and in (iii) subgroup 5, including North American, French and southwestern US cvs. As to subgroup 5, the Meridiano haplotype was observed in Edmore, a key founder of many North American and French cvs. and in seven of its derivatives, as well as in the Desert Durum® cv. West Bred 881.

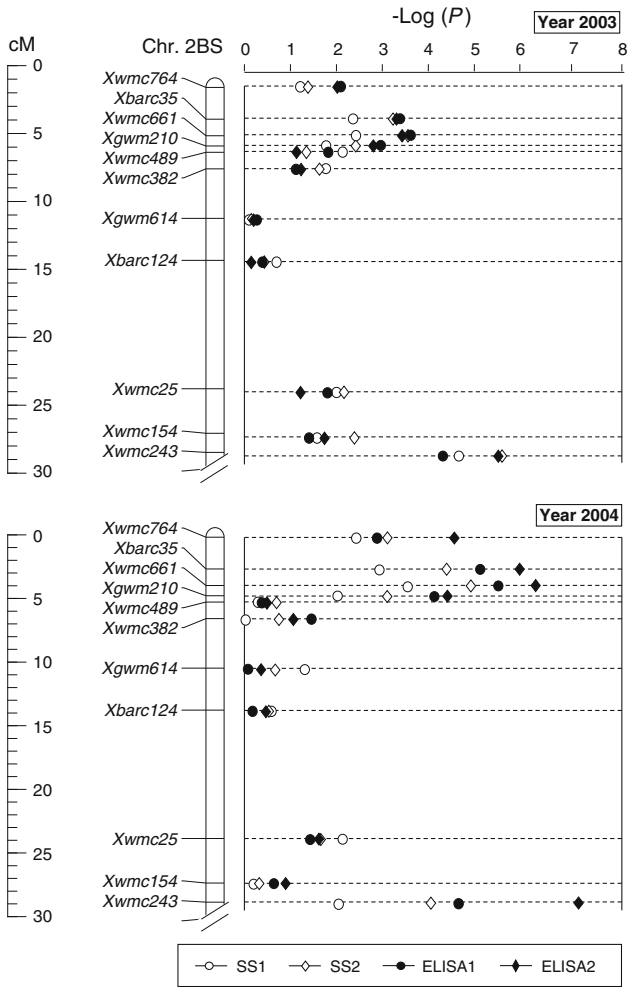

Fig. 5 Association between SSRs and SBCMV-response traits across the distal portion of chromosome 2BS harboring *QSbm.ubo-2BS*. Results refer to a germplasm panel of 111 elite durum wheat accessions evaluated for symptom severity (SS) and virus concentration (ELISA) in four scoring/sampling dates in 2003 and 2004. Association levels are reported as −Log (*P*)

It should be noted that the resistant parent of the RIL population, cv. Meridiano (pedigree Simeto/WB881//Duilio/F21), inherited the resistant allele from either Duilio or WestBred 881, important founders in sub-groups 2 and 5, respectively. Equally noteworthy is that the North American cv. Vic (pedigree Edmore/Ward) and the derived Italian cv. Ionio (pedigree Lira/Vic), while showing high levels of SBCMV resistance, presented a non-diagnostic haplotype at *Xbarc35-Xwmc661-Xgwm210*. However, both cvs. showed the A_{Mer} allele at the flanking marker *Xwmc489* as well as at *Xwmc243*.

Discussion

A thorough phenotypic and molecular characterization of a medium-size RIL population showing a marked transgressive segregation for SS and ELISA values allowed us to establish that the genetic control of SBCMV response in durum wheat is not exclusively based on major loci, but also on several loci having small effects. Indeed, in the M × C mapping population, SBCMV resistance was found to be controlled by a major QTL (*QSbm.ubo-2BS*) as well as by at least 11 minor QTLs spread across the genome. Such results explain the continuous range of SBCMV responses observed for durum wheat cvs. and suggest that resistance to SBCMV in the elite wheats has a more complex inheritance pattern than that hitherto reported in the literature for common wheat. It should be noted that an analogous, complex inheritance pattern has been reported for two other rod-shaped, cereal-infecting viruses transmitted by *P. graminis*, namely *Rice stripe necrosis virus* (RSNV, Gutiérrez et al. 2010) and *Oat golden stripe virus* (OGSV, Walker et al. 1998).

Role of *QSbm.ubo-2BS* in durum wheat

In the M × C durum population, the favorable allele at *QSbm.ubo-2BS* was found to have a major impact on SBCMV resistance as evidenced by its marked and significant effects on SS and ELISA values, as well as on physiological traits, such as NDVI and SPAD, and on grain yield and its components. Although the presence of the susceptible allele at *QSbm.ubo-2BS* caused a major drop in fertility (determined in the early phases of vegetative growth) accounting for most of the observed yield loss, other yield components such as TKW (determined in the last phases of the crop cycle) were also affected, probably as a result of the persistence of mosaic on leaves. The noticeable effects of *QSbm.ubo-2BS* on SBCMV response were confirmed by AM analysis across a germplasm collection of elite durum wheat accessions evaluated for SS and ELISA value only. On the other hand, it should be noted that AM analysis showed that *QSbm.ubo-2BS* accounted for less than 25% of the phenotypic variation for SBCMV response found in the elite durum germplasm collection, thus indicating the presence of other untagged QTLs.

QSbm.ubo-2BS was mapped in the M × C population in a 10-cM interval tagged by the SSR markers *Xwmc661*, *Xgwm210* and *Xbarc35* and by DArT markers wPt-2106 and wPt-1601, i.e., in a chr. interval which overlaps with that of the major SBCMV-resistance QTL *Sbm2* identified in the Avalon × Cadenza common wheat mapping population by Bayles et al. (2007). Whether *Sbm2-Cadenza* and *QSbm.ubo-2BS-Meridiano* have a common pre-hexaploidization origin or are independently derived alleles of the same locus, or (less likely) relate to distinct loci present on the same chr. region, could be answered only by further fine mapping and germplasm characterization studies, including the *T. dicoccum* and *T. dicoccoides* ancestors.

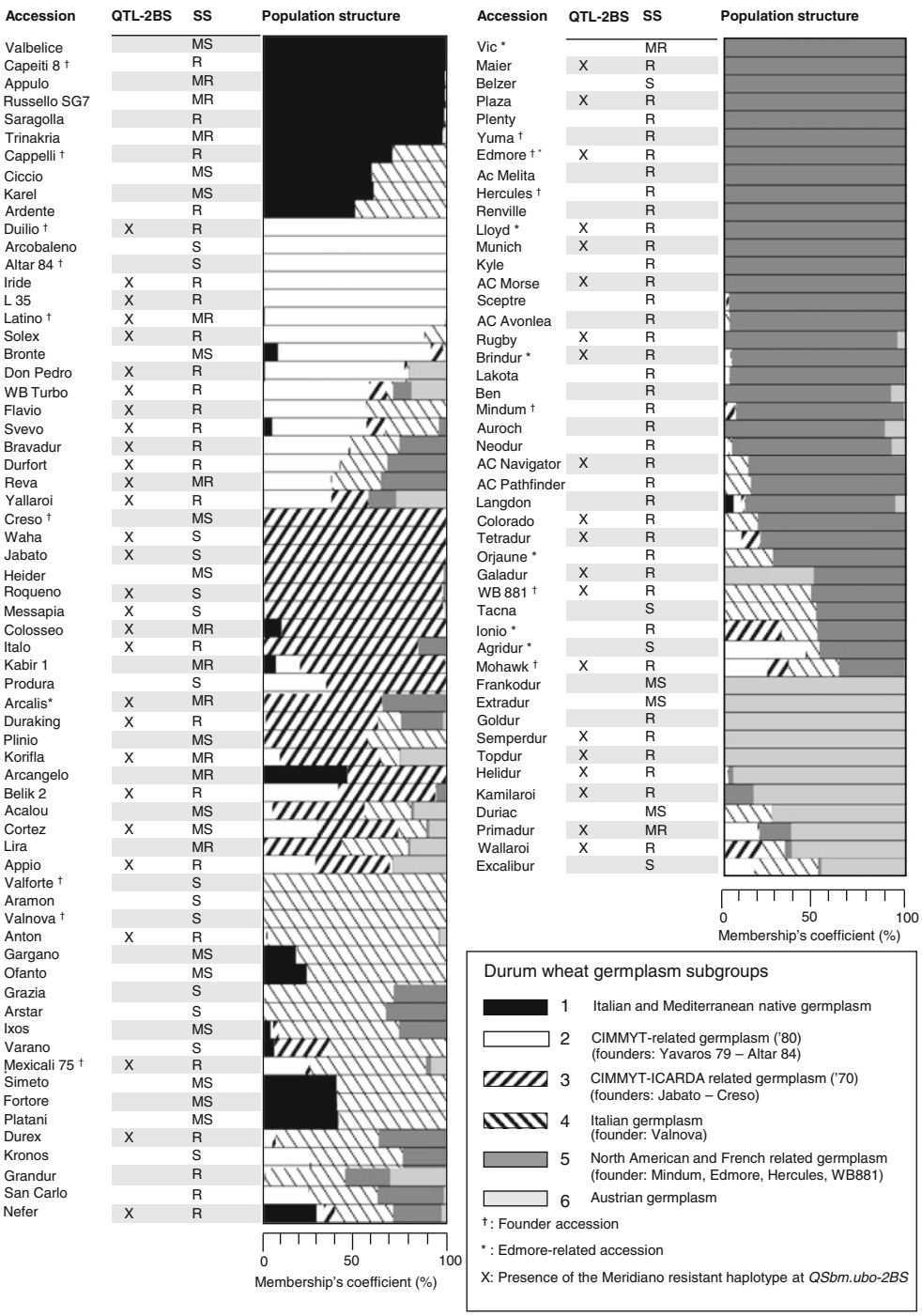

Fig. 6 Distribution of the SBCMV-resistance haplotype at *QSbm.ubo-2BS* in a germplasm collection of 111 elite durum wheat accessions reported according to the six main subgroups identified in the genetic structure analysis. Accessions are ranked according to their genetic relationships, based on the subgroup membership coefficients (%) that are represented with bars of different motifs. The presence of the resistant haplotype (identical to that present in the Meridiano parent of the RIL mapping population) at three critical markers (*Xwmc661*, *Xgwm210* and *Xbarc35*) has been indicated with '×' symbol. The SBCMV response of the accessions has been reported for symptom severity (SS) using a four-level scale (*S* susceptible, *MS* medium-susceptible, *MR* medium-resistant, and *R* (resistant)

The AM analysis, performed on the durum germplasm collection and based on the study of molecular variation at neutral markers in the *QSbm.ubo-2BS* region, allowed us to confirm the QTL position and to confine it to a more restricted (ca. 5 cM) chr. region flanked by a linkage block identified by the three SSRs: *Xbarc35-Xwmc661-Xgwm210* on the distal side and by *Xwmc489* on the proximal side. Moreover, the AM study carried out for the *QSbm.ubo-2BS* chr. region evidenced a strong association to SBCMV response also in the proximal end of the region, in correspondance with *Xwmc243*, ca. 20 cM distal from the LOD peak of *QSbm.ubo-2BS*. Conversely, no significant SBCMV effect was detected at *Xwmc243* in the RIL population. It should be noted that, in the AM study, the Meridiano allele at *Xwmc243* was in significant LD with the resistance-tagging haplotype at *QSbm.ubo-2BS*, thus allowing for the detection of highly significant associations with the resistant response. Considering that the genetic

distance between the two marker loci associated with resistance is higher as compared to the average LD extent observed in the region (5 cM) and that the resistance alleles/haplotypes are highly represented in the diverse germplasm pools (subgroups of accessions), the chr. region near *Xwmc243* could actually harbor an additional genetic factor related to SBCMV resistance, not segregating in the RIL population. Further molecular studies in the region with diverse genetic materials should clarify the genetic basis of the observed association.

Role of additional genetic factors

Once the sizeable and masking effect of *QSbm.ubo-2BS* was removed by restricting the analysis to a subset of 85 lines with the molecular haplotype at the *Xbarc35*–wPt2106–wPt1601 interval identical to the susceptible parent, a total of 11 minor QTLs (including the low-confidence ones) affecting SBCMV response were detected in the M × C population. The detection of chr. regions with minor effects in the M × C population strongly suggests that further minor QTLs could be easily identified in durum wheat. Since durum (AABB) and common (AABBDD) wheat are closely related species, it is likely that minor genes are widespread in common wheat too.

As to the nature of the minor QTLs affecting SBCMV response and here detected for the first time, they could not be necessarily related to direct plant-defense mechanisms per se, i.e., at least some of them can merely reflect the effects of specific morphophysiological plant characters interfering with SBCMV infection. *QSbm.ubo-5A*, for instance, mapped very close to *VRN-A1*, a locus controlling the vernalization requirement. i.e., the onset and duration of all the wheat phenological stages from tillering to maturity; thus, the differences in plant development due to the allele present at the *VRN-A1* locus could have indirectly affected the time course of SBCMV infection in the RILs. Similar interactions between SBCMV and plant characters of diverse nature may be envisaged; in fact, SBCMV is known to infect the root of wheats at a very early stage and to persist in their aerial parts well beyond the heading stage (Rubies-Autonell and Vallega 1991; Budge et al. 2008a; Vallega et al. 2006).

Outcomes for breeding

The allele associated with the SBCMV-resistant response at each of the four SSRs (and thus the corresponding resistant tagging haplotype) most closely associated with *QSbm.ubo-2BS* (*Xbarc35*, *Xwmc661*, *Xgwm210* and *Xwmc489*) was by far the most frequent in the germplasm collection. These findings, considered together with the high mutation rate of SSRs and the richness in allelic variation at the specific SSRs spanning the *QSbm.ubo-2BS* region, seem to indicate that a moderate selective sweep has occurred in the modern germplasm as a result of deliberate or indirect breeders' choices. In any case, it should be noted that the resistance-tagging haplotype was present in all the modern germplasm subgroups and in various breeding lineages, particularly in those that were selected in cultivation areas known to be subjected to the disease, or for worldwide adaptation. Based on the close association with the reported SSR markers, the resistance allele at *QSbm.ubo-2BS* can be easily transferred to other durum wheat breeding materials using marker-assisted selection.

It should be noted that *QSbm.ubo-2BS* proved to be sufficient to confer a high SBCMV-resistance level in a moderately susceptible genetic background, but not immunity to this virus. Indeed, RILs carrying also favorable alleles at 3–5 minor QTLs, in addition to the resistance allele at *QSbm.ubo-2BS*, showed clear signs of SBCMV infection. An incomplete resistance to SBCMV has been reported (Bayles et al. 2007) also for common wheat genotypes carrying the resistance allele at two major QTLs (*Sbm2*, homologous to *QSbm.ubo-2BS*, and *Sbm1,* on chr. 5DL).

The SBCMV-response distributions of the 85 M × C RILs not harboring the Meridiano resistant allele, together with the estimated R^2 and the additive effect values of the minor QTLs, suggested that lines with phenotypes markedly more resistant than the susceptible parent could be obtained based on cumulated resistance alleles at the minor QTLs only. However, due to their relatively low effects, marker-assisted selection strategies targeted to such minor QTLs cannot be envisaged, with the exception of genomic selection (Heffner et al. 2009); traditional phenotypic selection could nevertheless, in some cases, take advantage of the segregation of such QTLs.

Conclusions

Bi-parental linkage mapping analysis allowed us to establish that SBCMV resistance in the M × C durum wheat cross is governed by a complex genetic system, i.e., by a major QTL (*QSbm.ubo-2BS*) contributed by cv. Meridiano and by numerous minor QTLs spread across the genome and contributed by both parents. The numerous minor QTLs identified contributed to explain the continuous range of SBCMV responses reported for durum wheat cvs. tested in this as well as in previous studies (Rubies-Autonell et al. 2009). More in general, our results clearly demonstrate that in durum wheat, resistance to SBCMV is governed by a markedly more complex inheritance pattern than that hitherto reported in studies on common wheat.

Bi-parental linkage and AM analyses concurrently indicated that *QSbm.ubo-2BS* was located in a restricted interval of the distal telomeric region of chr. 2BS. Accessions with the resistance-tagging haplotype at the *QSbm.ubo-2BS* region (all characterized by an SBCMV-resistant phenotype) were detected at a relatively high frequency in the panel across subgroups of different origin, thus suggesting that the resistant haplotype was most probably subjected to positive selection in the durum germplasm. However, the relatively low portion of the phenotypic variation for SBCMV response in the elite durum germplasm explained by *QSbm.ubo-2BS* suggested the presence of other untagged QTLs. The identification of *QSbm.ubo-2BS* is relevant for durum wheat breeding because the exploitation of the resistance allele at this major QTL can be effectively carried out by MAS. Finally, the location of *QSbm.ubo-2BS* in the very distal chr. 2BS region, which according to Conley et al. (2004) is a gene-rich, highly recombinogenic region, is favorable to fine mapping and positional cloning of the causative gene, particularly by exploiting a comparative mapping strategy using the rice and *Brachypodium* sequenced genomes.

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References

Barbosa MM, Goulart LR, Prestes AM, Juliatti FC (2001) Genetic control of resistance to soil-borne wheat mosaic virus in Brazilian cultivars of *Triticum aestivum* L. Thell. Euphytica 122:417–422

Bass C, Hendley R, Adams MJ, Hammond-Kosack KE, Kanyuka K (2006) The *Sbm1* locus conferring resistance to soil-borne cereal mosaic virus maps to a gene-rich region on 5DL in wheat. Genome 49:1140–1148

Bayles R, O'Sullivan D, Lea V, Freeman S, Budge G, Walsh K (2007) Controlling soil-borne cereal mosaic virus in the UK by developing resistant wheat cultivars. HGCA Project 2616. HGCA Crop Research News 32: Project Report no. 418

Bonnefoy M, Boursereau J, Chesneau R (1994) Comportment des variétés face aux virus de la mosaique du blé et de la mosaique jaune du blé. Proceedings, Workshop on Mosaics of cereals transmitted by Polymyxa graminis Led., Blois, France, 7–8 April 1994, 57–62

Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES (2007) TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics 23:2633–2635

Breseghello F, Sorrells ME (2006) Association mapping of kernel size and milling quality in wheat (*Triticum aestivum* L.) cultivars. Genetics 172:1165–1177

Budge GE, Loram J, Donovan G, Boonham N (2008a) RNA2 of *Soil-borne cereal mosaic virus* is detectable in plants of winter wheat grown from infected seeds. Eur J Plant Pathol 120:97–102

Budge GE, Ratti C, Rubies-Autonell C, Lockley D, Bonnefoy M, Vallega V, Pietravalle S, Henry CM (2008b) Response of UK winter wheat cultivars to *Soil-borne cereal mosaic* and *Wheat spindle streak mosaic viruses* across Europe. Eur J Plant Pathol 120:259–272

Canova A (1966) Ricerche sulle malattie da virus delle graminaceae. III *Polymyxa graminis* Led. vettore del mosaico del frumento. Phytopatol Medit 5:53–58

Chao S, Zhang W, Dubcovsky J, Sorrells M (2007) Evaluation of genetic diversity and genome-wide linkage disequilibrium among US wheat (*Triticum aestivum* L.) germplasm representing different market classes. Crop Sci 47:1018–1030

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963–971

Clark MF, Adams AN (1977) Characteristics of the microplate method of enzyme-linked immunosorbent assay for detection of plant viruses. J Virol Meth 34:475–483

Clover G, Wright D, Henry C (1999) Occurrence of *Soil-borne wheat mosaic virus* (SBWMV) in the United Kingdom. Proceedings, fourth symposium of the international working group on plant viruses with fungal vectors. Monterey, USA, 5–8 October 1999, 105–108

Comadran J, Thomas WT, van Eeuwijk FA, Ceccarelli S, Grando S, Stanca AM, Pecchioni N, Akar T, Al-Yassin A, Benbelkacem A, Ouabbou H, Bort J, Romagosa I, Hackett CA, Russell JR (2009) Patterns of genetic diversity and linkage disequilibrium in a highly structured *Hordeum vulgare* association-mapping population for the Mediterranean basin. Theor Appl Genet 119:175–187

Conley EJ, Nduati V, Gonzalez-Hernandez JL, Mesfin A, Trudeau-Spanjers M, Chao S, Lazo GR, Hummel DD, Anderson OD, Qi LL, Gill BS, Echalier B, Linkiewicz AM, Dubcovsky J, Akhunov ED, Dyorak J, Peng JH, Lapitan NLV, Pathan MS, Nguyen HT, Ma XF, Miftahudin, Gustafson JP, Greene RA, Sorrells ME, Hossain G, Kalavacharla V, Kianian SF, Sidhu K, Dijbirligi M, Gill KS, Choi DW, Fenton RD, Close TJ, McGuire PE, Qualset CO, Anderson JA (2004) A 2600-locus chromosome bin map of wheat homoeologous group 2 reveals interstitial gene-rich islands and colinearity with rice. Genetics 168:625–637

Desiderio E, Belocchi A, D'Egidio MG, Fornara M, Cecchi V, Cecchini C (2007) Semina 2007: quali varietà di frumento duro scegliere. L'Informatore Agrario 34:5–6

Diao A, Chen J, Ye R, Zheng T, Yu S, Antoniw JF, Adams MJ (1999) Complete sequence and genome properties of *Chinese wheat mosaic virus*, a new furovirus from China. J Gen Virol 80:1141–1145

Dubey SN, Brown CM, Hooker AL (1970) Inheritance of field reaction to soil borne wheat mosaic virus. Crop Sci 10:93–95

Ersoz ES, Yu J, Buckler ES (2007) Applications of linkage disequilibrium and association mapping in crop plants. In: Varshney RK, Tuberosa R (eds) Genomics assisted crop improvement, vol 1: genomics approaches and platforms. Springer, Dordrecht, pp 97–119

Estes AP, Brakke MK (1966) Correlation of *Polymyxa graminis* with transmission of *Soil-borne wheat mosaic virus*. Virology 28:772–774

Eujayl ME, Sorrells M, Baum P, Wolters W, Powell W (2002) Isolation of EST-derived microsatellite markers for genotyping the A and B genomes of wheat. Theor Appl Genet 10:399–407

Farnir F, Coppieters W, Arranz JJ, Berzi P, Cambisano N, Grisart B, Karim L, Marcq F, Moreau L, Mni M, Nezer C, Simon P, Vanmanshoven P, Wagenaar D, Georges M (2000) Extensive

genome-wide linkage disequilibrium in cattle. Minimal linkage disequilibrium in human Xq25 and Xq28. Genome Res 10:220–227

Giunta F, Motzo R, Pruneddu G (2007) Trends since 1900 in the yield potential of italian-bred durum cultivars. Eur J Agro 27:12–24

Gutiérrez AG, Carabalí SJ, Giraldo OX, Martínez CP, Correa F, Prado G, Joe Tohme J, Lorieux M (2010) Identification of a rice stripe necrosis virus resistance locus and yield component QTLs using *Oryza sativa* × *O. glaberrima* introgression lines. BMC Plant Biol 10:6. doi:10.1186/1471-2229-10-6

Hall MD, Brown-Guedira G, Klatt A, Fritz AK (2009) Genetic analysis of resistance to soil-borne wheat mosaic virus derived from *Aegilops tauschii*. Euphitica 169:169–176

Heffner EL, Sorrels ME, Jannink JL (2009) Genomic selection for crop improvement. Crop Sci 49:1–12

Jesewska M (1994) Identification and some properties of wheat soil-borne mosaic virus isolated in Poland. Phytopathol Polon 8:97–102

Kanyuka K, Ward E, Adams MJ (2003) *Polymyxa graminis* and the cereal viruses it transmits: a research challenge. Mol Plant Pathol 4:393–406

Korzun V, Röder MS, Wendehake K, Pasqualone A, Lotti C, Ganal MW, Blanco A (1999) Integration of dinucleotide microsatellites from hexaploid bread wheat into a genetic linkage map of durum wheat. Theor Appl Genet 98:1202–1207

Kucharek TA, Walker JH (1974) The presence of and damage caused by Soil-borne wheat mosaic virus in Florida. Plant Dis Report 58:763–765

Kühne T (2009) Soil-borne viruses affecting cereals—known for long but still a threat. Virus Res 141:174–183

Maccaferri M, Sanguineti MC, Donini P, Tuberosa R (2003) Microsatellite analysis reveals a progressive widening of the genetic basis in the elite durum wheat germplasm. Theor Appl Genet 107:783–797

Maccaferri M, Sanguineti MC, Tuberosa R (2005) Analysis of linkage disequilibrium in a collection of elite durum wheat genotypes. Mol Breeding 15:271–289

Maccaferri M, Sanguineti MC, Natoli V, Araus Ortega JL, Ben Salem M, Bort J, Chenenaoui C, De Ambrogio E, Garcia del Moral L, Demontis A, El-Ahmed A, Maalouf F, Machlab H, Moragues M, Motawaj J, Nachit M, Nserallah N, Ouabbou H, Royo C, Tuberosa R (2006) A panel of elite accessions of durum wheat (*Triticum durum* Desf.) suitable for association mapping studies. Plant Genet Resour 4:79–85

Maccaferri M, Stefanelli S, Rotondo F, Tuberosa R, Sanguineti MC (2007) Relationships among durum wheat accessions. I. Comparative analysis of SSR, AFLP, and phenotypic data. Genome 50:373–384

Maccaferri M, Mantovani P, Tuberosa R, DeAmbrogio E, Giuliani S, Demontis A, Massi A, Sanguineti MC (2008a) A major QTL for durable leaf rust resistance widely exploited in durum wheat breeding programs maps on the distal region of chromosome arm 7BL. Theor Appl Genet 117:1225–1240

Maccaferri M, Sanguineti MC, Corneti S, Araus Ortega JL, Ben Salern M, Bort J, DeAmbrogio E, Garcia del Moral L, Demontis A, El-Ahmed A, Maalouf F, Machlab H, Martos V, Moragues M, Motawaj J, Nachit M, Nserallah N, Ouabbou H, Royo C, Slama A, Tuberosa R (2008b) Quantitative trait loci for grain yield and adaptation of durum wheat (*Triticum durum* Desf.) across a wide range of water availability. Genetics 178:489–511

Maccaferri M, Ratti C, Rubies-Autonell C, Tuberosa R, Demontis A, Massi A, Stefanelli S, Vallega V, Sanguineti MC (2008c) Mapping genetic factors for resistance to *Soil-Borne cereal mosaic virus* (SBCMV) in durum wheat. 11th international wheat genetics symposium. Brisbane, Australia. 24–29 August, 2008

Mantovani P, Maccaferri M, Sanguineti MC, Tuberosa R, Catizone I, Wenzl P, Thomson B, Carling J, Kilian A (2008) An integated DArT-SSR linkage map of durum wheat. Mol Breeding 22:629–648

Marti J, Bort J, Slafer GA, Araus JL (2007) Can wheat yield be assessed by early measurements of normalized difference vegetation index? Ann Appl Biol 150:253–257

McKinney HH (1923) Investigations of the rosette disease of wheat and its control. J Agric Res 23:771–800

Merkle OG, Smith EL (1983) Inheritance of resistance to soil-borne mosaic in wheat. Crop Sci 23:1075–1076

Modawi RS, Heyne EG, Brunetta D, Willis WG (1982) Genetic studies of field reaction to wheat soil-borne mosaic virus. Plant Dis 66:1183–1184

Nakagawa M, Soga Y, Watanabe S, Gocho H, Nishio K (1959) Genetical studies on the wheat mosaic virus II. Genes affecting the inheritance of susceptibility to strains of yellow mosaic virus in varietal crosses of wheat. Jpn J Breed 9:118–120

Narasimhamoorthy B, Gill BS, Fritz AK, Nelson JC, Brown-Guedira GL (2006) Advanced backcross QTL analysis of a hard winter wheat × synthetic wheat population. Theor Appl Genet 112:787–796

Perovic D, Forster J, Devaux P, Hariri D, Guilleroux M, Kanyuka K, Lyons R, Weyen J, Feuerhelm D, Kastirr U, Sourdille P, Röder M, Ordon F (2009) Mapping and diagnostic marker development for Soil-borne cereal mosaic virus resistance in bread wheat. Mol Breed 23:641–653

Ratti C, Pisi A, Vallega V, Rubies Autonell C (2005) Molecular characterization of Italian *Soil-borne cereal mosaic virus* isolates. Parasitica 61:11–16

Ratti C, Rubies-Autonell C, Maccaferri M, Stefanelli S, Sanguineti MC, Vallega V (2006) Reaction of 111 cultivars of *Triticum durum* Desf. from some of the world's main genetic pools to soil-borne cereal mosaic virus. J Plant Dis Protect 113:145–149

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Rohlf FJ (1997) NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System Version 2.1. Exeter Software, Setauket, New York

Rubies-Autonell C, Vallega V (1987) Observations on a mixed Soil-borne wheat mosaic virus and Wheat spindle streak mosaic virus infection in durum wheat (*Triticum durum* Desf.). J Phytopathol 119:111–121

Rubies-Autonell C Vallega V (199l) Studies on the development and interaction of *Soil-borne wheat mosaic virus* and *Wheat Spindle streak mosaic virus*. In Biotic interactions and soil-borne diseases, Proc. First Congress of the European Foundation of Plant Pathology, Wageningen, Netherlands (Eds. ABR Beemster, GJ Bollen, M Gerlach, MA Ruissen, B.). Schippers and A. Tempel. Elsevier Scientific Publishers, Amsterdam, pp 107–112

Rubies-Autonell C, Vallega V, Ratti C (2003) Reactions of cultivars of common wheat (*Triticum aestivum* L.) to soilborne wheat mosaic virus in northern Italy during 1996–1997. J Plant Dis Protect 110:332–336

Rubies-Autonell C, Ratti C, Vallega V (2009) Indexed data for comparing the reaction to cereal soil-borne mosaic virus of durum wheat cultivars assayed in different seasons. In: Rush CM (ed) Proc. of the seventh symposium of the international working group on plant viruses with fungal vectors (IWGPVFV). Quedlingburg, Germany

Shaalan M, Heyne EG, Sill WH (1966) Breeding wheat for resistance to soil borne wheat mosaic virus, wheat streak-mosaic virus, leaf rust, stem rust, and bunt. Phytopathology 56:664–669

Shirako Y, Suzuki N, French RC (2000) Similarity and divergence among viruses in the genus *Furovirus*. Virology 270:201–208

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for breeding wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114

Song QJ, Shi JR, Singh S, Fickus EW, Costa JM, Lewis J, Gill BS, Ward R, Cregan PB (2005) Development and mapping of microsatellite (SSR) markers in wheat. Theor Appl Genet 110:550–560

Torrance L, Koenig R (2005) Genus Furovirus. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA (eds) Virus taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, London, pp 1027–1032

Vaïanopoulos C, Legrève A, Moreau V, Bragard C (2009) Broad-spectrum detection and quantitation methods of Soil-borne cereal mosaic virus isolates. J Virol Methods 159(2):227–232

Vallega V (2004) Soil-borne cereal mosaic. In: Lapierre H, Signoret PA (eds) Viruses, virus diseases of Poaceae (Gramineae). INRA, Paris, pp 612–613

Vallega V, Rubies Autonell C (1985) Reactions of Italian *Triticum durum* cultivars to soilborne wheat mosaic. Plant Dis 69:64–66

Vallega V, Rubies-Autonell C, Turina M, Ratti C, Contoli S (1999) Reactions to SBWMV of durum wheat cultivars grown in northern Italy during 1995–96. J Plant Dis Protect 106:284–290

Vallega V, Rubies-Autonell C, Ratti C (2006) Resistance to accumulation of *Soil-borne cereal mosaic virus* in eight cultivars of *Triticum durum* Desf. Parasitica 62:79–96

Van Ooijen JW (2006) JoinMap® 4, Software for the calculation of genetic linkage maps in experimental populations. Kyazma BV, Wageningen

Varshney RK, Tuberosa R (2007) Genomics-assisted crop improvement: an overview. In: Varshney RK, Tuberosa R (eds) Genomics assisted crop improvement, vol. 1: genomics approaches and platforms. Springer, Dordrecht, pp 1–12

Walker SL, Leath S, Murphy JP, Lommel SA (1998) Selection for resistance and tolerance to oat mosaic virus and oat golden stripe virus in hexaploid oats. Plant Dis 82:423–427

Weir BS (1996) Genetic data analysis II. Sinauer Associates, Sunderland

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Res 14:415–421