Mapping genetic factors for resistance to *Soil-Borne cereal mosaic virus* (SBCMV) in durum wheat

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ABSTRACT

Genetic analysis of SBCMV resistance in durum wheat was carried out using a population of 181 recombinant inbred lines (RILs) obtained from Meridiano (moderately resistant) × Claudio (moderately susceptible). The RILs were characterized for SBCMV response in the field under severe and uniform SBCMV infection during 2007 and 2008 and profiled with SSR and DArT markers. A wide range of disease reaction (as estimated by symptoms and DAS-ELISA) was observed. Most 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 *Xwmc243*, with the favourable allele contributed by Meridiano. QTLs with minor effects on SBCMV-response were also detected. Consistent with the observed transgressive segregation, both parents contributed resistance alleles. *QSbm.ubo-2BS* significantly affected grain yield and test weight of the grains.

INTRODUCTION

Soil-borne cereal mosaic virus (SBCMV), a Furovirus transmitted by *Polymyxa graminis* Led., is responsible for an important disease of wheat (Koenig and Huth, 2000) and it is widespread in the main wheat growing areas of the world. Although most of the durum wheat cultivars (cvs.) grown in Italy and in the Mediterranean region are characterized by a disease response ranging from susceptible to medium-resistant, valuable sources of resistance have been identified in the cultivated durum germplasm (Rubies et al, 2006; Ratti et al, 2006).
Recently, a main locus for disease resistance (*Sbm1*) has been identified in hexaploid wheat, on chromosome 5DL (Bass et al, 2006). However, this source of genetic resistance is not readily available to durum wheat breeders, due to its location on the D genome. To date, no specific genetic analysis of SBCMV resistance has been reported in durum wheat.
The results of the QTL analysis for SBCMV resistance carried out on a durum wheat mapping population are presented and discussed.

MATERIALS AND METHODS

A population of 181 recombinant inbred lines (RILs), developed by Società Produttori Sementi (Bologna, Italy) from the cross between cvs. Meridiano (Simeto/WB881//Duilio/F21) and Claudio (CIMMYT'selection/Durango//IS139b/Grazia) was considered. Meridiano and Claudio were released in Italy during 1999 and are widely cultivated across Europe. Meridiano is moderately resistant to SBCMV, with mild symptoms even under high infection levels, while Claudio is moderately susceptible (Vallega et al, 2002). Seeds of the RILs were bulked in the F_7 generation.
The RILs were tested for SBCMV-response during 2007 and 2008 in Cadriano, Northern Italy (44°35'N 11°27'E, Po valley), in a field showing a severe and uniform SBCMV infection. For each experiment, the field layout was a randomized complete block design with two replications. Each plot (2.4 m^2) included eight 2.0 m long rows. Five plots of each parent as well as of the highly susceptible check cv. Grazia were included into each replication.
In both years, symptom severity (SS) was scored at different plant growth stages, using a 0 (no symptoms) to 4 (severe mottling and stunting) scale (Vallega and Rubies-Autonell, 1985). For the sake of conciseness, we report the results of the mid-end of tillering and first-node stage scores (SS1 and SS2, respectively) only. For the 2007 experiment, the following traits were also available: virus concentration determined twice (ELISA1 and ELISA2) on bulk samples of leaves collected at the same plant stages as SS1 and SS2 from 14 randomly chosen plants per plot, grain yield normalized at 13% moisture (GY, q/ha) and test weight (TWT, kg/hl). Virus concentration was measured using DAS (double antibody sandwich) ELISA according to Clark and Adams (1977). SS data (x + 0.5) were square root- transformed prior to conduct ANOVA and QTL analysis.
The molecular genetic map was assembled using the wheat simple sequence repeat (SSR) sets available on the GrainGenes database, togheter with a private WMS set provided by Dr. Martin Ganal (TraitGenetics, Gatersleben, Germany) and a set of DArT markers generated with the durum DArT v. 2.0 *Pst*I/*Taq*I service by Triticarte Pty Ltd (Yarralumla, Australia). DArT markers mapped from hexaploid wheat clones are

indicated with the "wPt-" notation, while durum DArT markers are identified by their numeric code only. A provisional linkage map was constructed with 414 markers (98 SSRs and 316 DArT markers), grouped at high LOD threshold (≥ 6) into 41 linkage groups and covering a total of 1856 cM. Linkage groups were identified using JoinMap 4.0 (Stam, 1993). Marker ordering and mapping (Haldane's mapping function) was performed for each linkage group using Record (van Os et al. 2005) and JoinMap 4.0 programs. A subset of 213 markers selected for high informativeness and even genetic distribution (intermarker distance ≥ 3 cM) was used for QTL analysis. 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). "Model 6 standard analysis" with up to 5 control markers and a window size of 10 cM was used in the CIM analysis. Based on permutations, only QTLs with LOD peaks > 3 were declared as significant QTLs.

RESULTS AND DISCUSSION

In both seasons, infection level throughout the field experiment was quite uniform as indicated by the limited variability (data not reported) among the replicated plots of the check cv. Grazia (highly susceptible) for SS and ELISA values.

ANOVA indicated that the differences among RILs were significant (*P* 0.01) for all the considered traits (data not reported). Table 1 summarizes the results of the two-year phenotypic evaluation for Meridiano, Claudio, Grazia and the RILs. The RILs exhibited transgressive segregation in both directions, indicating complex inheritance of SBCMV-response with resistance alleles contributed by both parents. Grain yield of the RILs (2007 data) ranged from 2.8 to 69.8 q/ha and was severely affected by infection as evidenced by the high values of the correlation coefficients between GY and both SS1 and SS2 (*r* -0.83 and -0.93, respectively, *P* 0.001) as well as ELISA1 and ELISA2 values (*r* -0.72 and -0.76, respectively, *P* 0.001). The negative effect of SBCMV was also noticed on TWT (data not reported).

Single-marker analysis using all 414 markers segregating among the RILs showed that a relatively limited number of markers, grouped in a few chromosome (chr.) regions, were involved in the SBCMV-response. The most relevant region significantly associated to SS1 and SS2 in both years and ELISA1 and ELISA2 in 2007 was identified in a 30 cM wide interval located in the distal end of the 2BS linkage group. Five markers encompassed this region: four DArT markers (117438, wPt-5738, wPt-2106 and 381522) located in the 2BS telomeric region within a 5 cM distance and one SSR marker (*Xwmc243*) located at a 30.8 cM distance. The map position of the DArT markers was validated after inspection of co-linearity with the hexaploid-wheat DArT-based genetic map (http://www.triticarte.com.au/content/publications.html) and with a proprietary DArT- and SSR- based durum linkage map (Mantovani et al., 2008). The LOD values at the QTL peak (wPt-2106) were very high (> 40) for most of the SBCMV-response traits. The favourable allele conferring the resistant response was inherited from Meridiano. The same chr. region significantly affected also GY and TWT.

Seven additional chr. regions showing significant marker-trait associations (*P* values of linear regression ≤ 0.01) across SBCMV-response traits (i.e. SS and ELISA values) and years were located on chr. 1BL (378539 and wPt-6142), 3BS centromeric region (wPt-8686, *Xwmc43*, wPt-1159, *Xgwm685* and wPt-5390), 4AL proximal region (*Xwmc161*, 379716, wPt-7289), 5AS (*Xgwm443.1*), 5AL (348667, 349142, *Xwmc524*), 5BL (*Xbarc243*) and 7BL (wPt-3533, wPt-1330, 117233, *Xgwm132.2*). At these chr. regions, each showing a limited genetic effect, the favourable alleles were contributed by both the resistant and the susceptible parents (the allele increasing the resistance response was contributed by Meridiano at the 1BL, 3BS, 4AL and 5AL chromosome regions, and by Claudio at the 5AS, 5BL and 7BL chromosome region, thus supporting the observed transgressive segregation. The QTLs identified in the distal regions of chrs. 5AL and 5BL in our durum population could represent the homoeologous copies of *Sbm1* detected by Bass et al (2006) in bread wheat.

Composite interval mapping confirmed the presence of a major QTL (*QSbm.ubo-2BS*) for SBCMV-response located on the distal end of chr. 2BS. The statistical features of this major QTL are reported in Table 2. At *QSbm.ubo-2BS*, the LOD peak values for all the SBCMV-response traits were comprised between 40 and 100 across the two years. The location of *QSbm.ubo-2BS* is coincident with that of the recently reported *Sbm2* locus, detected in the hexaploid wheat background (cv. Cadenza) in the 2BS chr. end (Bayles et al, 2007). In the CIM analysis, only two additional chr. regions (4AL and 7BL) out of the seven previously identified by single-marker regression showed significant LOD values; however, their effect was very limited. The same results were obtained when considering a subset of 77 RILs with a fixed haplotype homogeneous to Claudio (susceptible parent) at the major QTL region. Once a complete map from the Meridiano x Claudio mapping population is assembled, a more detailed analysis of the genetic control of SBCMV response will be carried out.

Our results indicated that the genetic control of SBCMV resistance in the durum mapping population Meridiano x Claudio is oligogenic, with a major QTL (*QSbm.ubo-2BS*) explaining most of the variability for the SBCMV-response traits. This finding is supported by the fact that most of the durum wheat genome has been explored by the markers herein analysed. However, it should be noted that the favourable allele carried by Meridiano at this major QTL does not provide complete resistance, as demonstrated by the mean symptom severity and ELISA values of the parental cultivar. This work integrates the results obtained up to now in hexaploid wheat, and highlights the importance of the 2BS telomeric chr. region in the control of resistance to SBCMV in wheat and its relevance for marker-assisted selection strategies.

Table 1. Mean phenotypic value and range of variation for the traits related to the SBCMV-response for 181 durum wheat RILs from the cross Meridiano x Claudio, as evaluated in field experiments under natural SBCMV infection for two years (2007 and 2008). Mean values of the two parents and the susceptible check Grazia are also reported.

Traits		RILs			Meridiano	Claudio	Grazia
		Mean	Range	h^2 (%)	Mean	Mean	Mean
2007							
SS1	(Visual rating)	1.29	0.00 – 3.25	88.3	0.06	1.93	3.18
SS2	(Visual rating)	1.22	0.00 – 3.12	97.6	0.06	2.53	3.43
ELISA1	(ELISA units)	1.15	0.14 – 1.82	64.3	0.90	1.44	1.47
ELISA2	(ELISA units)	1.22	0.08 – 1.98	78.2	1.37	1.69	1.79
GY	(q/ha)	34.1	2.8 – 69.8	89.2	55.6	18.7	9.9
TWT	(kg/hl)	72.4	63.5 – 76.6	61.6	72.8	72.4	64.8
2008							
SS1	(Visual rating)	1.67	0.00 – 3.75	84.7	0.27	2.28	3.45
SS2	(Visual rating)	1.44	0.00 – 3.25	93.3	0.33	2.18	3.47

Table 2. Main features of *QSbm.ubo-2BS,* the major QTL for SBCMV-response found in the chromosome 2BS distal region based on two-year data. Additive effect is reported as half of the difference between the mean phenotypic value of the RILs carrying the allele inherited from Claudio and that of the RILs with the Meridiano allele.

		2007						2008	
		SS1	SS2	ELISA1	ELISA2	GY	TWT	SS1	SS2
LOD peak	LOD units	61.3	100.4	39.3	32.6	63.6	6.4	61.7	97.9
Significance interval (LOD > 3)	cM	0 – 30	0 – 30	0 – 30	0 – 30	0 – 30	0 – 25	0 – 30	0 – 30
LOD peak position (from top of chr.)	cM	3	7	5	3	5	5	5	5
Explained phenotypic variance (R^2)	%	72.6	86.7	68.2	49.8	78.9	15.4	70.5	85.5
Additive effect *(a)*		+ 0.91	+ 1.22	+ 0.36	+ 0.37	- 18.2	- 1.0	+ 1.12	+ 1.21

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