Theor Appl Genet (2008) 117:1225–1240
DOI 10.1007/s00122-008-0857-5

ORIGINAL PAPER

A major QTL for durable leaf rust resistance widely exploited in durum wheat breeding programs maps on the distal region of chromosome arm 7BL

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Received: 26 January 2008 / Accepted: 28 July 2008 / Published online: 19 August 2008

Abstract A recombinant inbred line (RIL) population and a set of advanced lines from multiple crosses were used to investigate the leaf rust (*Puccinia triticina* Eriks.) resistance carried by the durum wheat cultivar Creso and its derivatives (Colosseo and Plinio). One hundred seventy-six RILs from the cross Colosseo × Lloyd were tested under artificial rust inoculation in the field. The response at the seedling stage was also investigated. A major QTL (*QLr.ubo-7B.2*) for leaf rust resistance controlling both the seedling and the adult open field based-response was mapped on 7BL, with the favourable allele inherited from Colosseo. *QLr.ubo-7B.2* showed R^2 and LOD peak values for the area under disease progress curve (AUDPC) equal to 72.9% and 44.5, respectively. The presence and location of *QLr.ubo-7B.2* was validated by a linkage disequilibrium-based test using two-year field data of 62 advanced lines from 21 crosses with Creso, Colosseo or Plinio as resistance donors. *QLr.ubo-7B.2* maps in a gene-dense region (7BL10-0.78-1.00) carrying several genes/QTLs in wheat and barley for resistance to rusts and other fungal diseases.

Communicated by J. Snape.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-008-0857-5) contains supplementary material, which is available to authorized users.

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Introduction

Leaf rust (*Puccinia triticina* Eriks.) is one of the most damaging foliar pathogens of wheat. Epidemics of rusts, sometimes caused by new pathogen races, frequently affect wheat grain production and quality throughout the world. Sources of genetic resistance are valuable to increase the sustainability of cereal production, from both economic and environmental standpoints (Reynolds and Borlaug 2006).

Genetics of leaf rust resistance has long been studied in wheat, especially hexaploid wheat (*Triticum aestivum* L.), where ca. 50 *Lr* genes have been identified (Kolmer 1996). A number of these genes have been characterised for differential reaction to single leaf rust isolates and mapped to wheat chromosome (chr.) arms (McIntosh et al. 1995; http://www.cdl.umn.edu/res_gene/wrl.html). Most of these genes belong to the race-specific gene class where the incompatible interaction is controlled by a relatively simple gene-for-gene recognition pattern (hypersensitive resistance). As a consequence, single R-genes are easily overcome by rapidly changing *Puccinia triticina* populations with the spread of new virulent pathotypes (Kolmer et al. 2007). Obtaining cultivars (cvs.) with durable resistance (see Johnson 1984, for a detailed description of the meaning of this term) is a major target for wheat geneticists, pathologists and breeders. For this purpose, two approaches have been suggested: (1) pyramiding more than two *Lr* genes, mainly through marker-assisted selection (Chelkowski and Stepien 2001), and (2) pursuing the genetic characterisation and mapping of durable resistance

factors. Many leaf rust resistance genes have been mapped in wheat during the past decade by means of linkage mapping using molecular markers and recombinant inbred populations (http://www.cdl.umn.edu/res_gene/wlr.html).

Although leaf rust is a major threat to durum wheat (*Triticum turgidum* L. var. *durum*) production worldwide (Pasquini et al. 1997; Singh and Rajaram 2002; Herrera-Foessel et al. 2006), a detailed analysis of the genetic bases of resistance has been undertaken only recently (Herrera-Foessel et al. 2005, 2007; Martinez et al. 2007). This is further complicated by the finding that virulence of durum leaf rust isolates is different from that of common wheat isolates (Ordoñez and Kolmer 2007a, b).

In this study, we investigated the genetic basis of the resistance to *Puccinia triticina* conferred by the durum cv. Creso, a very successful Italian cv. released in 1974. Creso was obtained by crossing a CIMMYT's advanced line with a semi-dwarf Cappelli mutant (Cp B14). Due to its positive characteristics for yield potential, gluten quality and leaf rust resistance, Creso has been largely used in breeding programs throughout the Mediterranean Basin (Scarascia Mugnozza 2005; De Vita et al. 2007). Creso has been a source of resistance to leaf rust in durum wheat under field conditions that has remained effective since 1975 in cultivation environments characterised by recurrent leaf rust epidemics (Pasquini and Casulli 1993; Martinez et al. 2007), thus fulfilling the basic requirement for being considered as a durable resistance, according to the definition provided by Johnson (1984). Several new cvs. directly derived from Creso were released by breeders throughout the 1980s and 1990s. The two Italian cvs. Plinio and Colosseo, released in 1988 and 1995, respectively, and tightly related to Creso as shown by the molecular profiles based on simple sequence repeat (SSR) and NBS-specific markers (Mantovani et al. 2006; Maccaferri et al. 2007a), showed the leaf rust resistance phenotype inherited from Creso (DeAmbrogio, personal communication; Martinez et al. 2007).

The objectives of this study were: (1) to investigate the genetic control of the resistance to leaf rust from the durum cv. Creso and its derivatives by evaluating the infection response of segregating populations at the seedling stage under controlled conditions and at the adult plant stage under field conditions, (2) to map the corresponding genetic determinants, and (3) to identify microsatellite markers to enhance the efficiency of selection for resistant genotypes.

Materials and methods

Plant materials

A population of 176 recombinant inbred lines (RILs, $F_{6:8}$) was produced through single-seed descent from the cross between two elite semi-dwarf cvs. (the Italian cv. Colosseo and the North American cv. Lloyd) by Società Produttori Sementi Bologna s.p.a. (PSB, Bologna, Italy). Pedigree information indicates a direct lineage between Colosseo (Mexa's mutant/Creso) and Creso (Yaktana54/Norin10//Brevor///*2Cappelli-63/4/*3Tehucan60//5/Cappelli B14). Lloyd (Cando/Edmore, North Dakota State University; Cantrell et al. 1984) belongs to the North American durum gene pool.

Lloyd, despite being a cv. known to harbour genes for seedling and adult resistance (Zhang and Knott 1993), shows a susceptible response to leaf rust under field conditions in Italy (DeAmbrogio, personal communication), while Colosseo is characterised by field resistance. Under the growing conditions of Southern Europe (autumn sowing), both parents of the mapping population are medium- to late-heading and maturing.

A panel of 62 $F_{6:8}$ lines from the breeding program of PSB and Sementi Samoggia Srl (Crevalcore, Italy) was used to further investigate and validate under field conditions the presence of the major gene for leaf rust resistance inherited from Creso and its derivatives. The advanced lines were selected from 21 different crosses (simple, three- and four-way crosses) based on the presence in their pedigree of Creso and/or one of its more recent direct derivatives (e.g. Colosseo and Plinio) known to carry the same durable leaf rust field resistance. No specific selection for resistance to leaf rust and other pathogens was applied during the line development. For each cross, one to seven independently-derived F_6 lines were considered for field and molecular analysis.

Field trials and phenotypic traits

The materials were evaluated in artificially inoculated field trials carried out at Argelato, Bologna (Po Valley, 44°39′03.52″N 11°20′34.47″E), Italy. The 176 Colosseo × Lloyd (C × L) RILs and their two parents were evaluated in 2006, while the advanced lines tracing back to Creso were evaluated in 2006 and 2007 together with Creso, Colosseo, Plinio and four leaf rust susceptible checks.

Field trials were sown in replicated plots arranged in a randomised complete block (RCB) design with three replications; each plot consisted of two 2.5 m-long and 0.15 m-apart rows, spaced 0.55 m between rows of adjacent plots. Two hundred germinating seeds per plot were sown in November of each year, which is the normal sowing date for the area. The parents of the RILs and/or check cvs. were repeated within the experimental blocks to assess the leaf rust infection homogeneity in the field trials. Before sowing, fields were fertilized with 90 kg/ha P_2O_5 and 30 kg/ha N. In both years, field emergence was high

and plants were well established before winter. During winter and early spring a total of 150 kg/ha N was distributed at three growth stages (Zadocks scale 13, 20 and 31, i.e. from leaf three unfolded to the first node detectable stages; Zadocks et al. 1974). The ordinary cultural practices were applied to control weeds and pests and to ensure optimum crop development. No fungicides were applied.

All plots in the field trials were artificially inoculated with a mixture of 16 isolates of *Puccinia triticina* collected during the 1999–2006 period from different durum varieties in different Italian locations, representing the main durum wheat-growing areas usually characterised by high levels of leaf rust epidemics. Six isolates (1, 5, 6, 7, 9 and 10) were collected in Southern Italy, mainly in the Puglia region, while the others have been collected from Northern Italy. Each of the isolates has been maintained on the specific susceptible cv. initially hosting the pathogen. The pathogenicity characterization of the 16 Italian leaf rust isolates is underway using the Thatcher differential isolines and a panel of durum wheat accessions (P. Mantovani et al., unpublished). In this respect, it should be noted that the characterization of leaf rust isolates in durum wheat is not as straightforward as in hexaploid wheat, due to the lack of a differential set of lines developed in a common susceptible genetic background; moreover, the *P. triticina* populations predominant in durum are characterized by a different pathogenicity as compared to those wide-spread in hexaploid wheat (Huerta-Espino and Roelfs 1989, 1992; Herrera-Foessel et al. 2007; Ordoñez and Kolmer 2007a, b).

Seedlings were grown under isolation in mini-tunnels in greenhouse and, when reaching the first-leaf stage, were inoculated with a mixture of talc and spores (6 to 1 ratio; v/v). During the first 24 h, tunnels were covered with a black plastic film; temperature and relative humidity were maintained at 18°C and 100%, respectively. After removing the black film, temperatures ranged from 20–22°C (day) to 16–18°C (night). After 14–16 days from inoculation, spores were collected in special glass tubes to be dehydrated. Five cycles of spore production were completed to produce the amount suitable for field inoculation.

Inoculation was carried out by spraying all plants with a water plus 1% Tween® 20 (Fluka, Buks, Germany) suspension of spores. Three inoculations were carried out starting from booting stage up to complete flowering (Zadocks scale from 39 to 69). During the three days following inoculation, water was sprayed with sprinklers onto the plants to maintain high moisture and enhance leaf rust spread.

All the genetic materials evaluated in the field trials were scored for reaction to leaf rust by visually estimating the percentage of infected leaf area according to the modified Cobb scale (Peterson et al. 1948); scoring began in each field trial when the reference susceptible parent Lloyd showed a 10% value of infected leaf area across the replicates within blocks. Three visual scores were recorded for the RIL experiment in 2006; two visual scores were recorded in 2006 and three in 2007 on the advanced lines related to Creso. In order to more precisely assess the percentage of leaf area infected by rust, visual scores were recorded separately for the flag leaf and for the lower portion of the canopy at the same time. These two scores were averaged to obtain an index of percentage of infected leaf area (leaf rust susceptibility index, LRS); for each field trial, the area under the disease progress curve (AUDPC, Shaner and Finney 1977) was then calculated as follows:

$$\text{AUDPC} = \sum_{i=1}^{n} [(\text{LRS}_i + \text{LRS}_{i+1})/2] \times (t_{i+1} - t_i)$$

where n indicates the number of assessment times (minimum two and maximum three), LRS indicates the leaf rust susceptibility index, and t the time in days from the first scoring.

In 2006, the RILs were also evaluated in an additional field trial carried out in the same location as the infected trial using an RCB design (three reps) and field practices as previously described; in this case, however, no inoculation was made and fungicides were applied to prevent leaf rust attacks.

Heading date (HD), mean kernel weight (TKW) and grain test weight (TW) were recorded in both inoculated and disease-protected RIL field trials.

Seedling tests

The seedling leaf rust response was evaluated under greenhouse condition using single leaf rust isolates. Each isolate was increased on its specific susceptible host cv. as previously described.

Preliminary experiments were conducted to test the two RIL parents Colosseo and Lloyd for the presence of differential responses to each of the 16 leaf rust isolates that were used in the open field inoculation mixture. Additionally, for each isolate, the corresponding hosting cv. together with Creso, the suceptible cv. Aconchi, and the accession UCRD05-2 (Aconchi background) carrying the translocation 1–23 from the 7E chr. of *Lophopyrum ponticum* (Podp.) Á. Löve with the resistance gene *Lr19* (Lukaszewski 2006) were considered. Based on the results of these experiments, four isolates were chosen to test a selected informative sub-sample of the C × L RILs; moreover, one of the selected isolate was used to evaluate the seedling response of all the RILs together with Creso and its resistant derivatives.

All experiments were carried out with three replications. The experimental unit consisted of 12 seedlings sown in a

single pot of a flat tray. Five days after sowing, seedlings of each tray were treated with 500 ml of a maleic hydrazide solution (1.25 mg/1,000 ml of water) to slow down growth and enhance spore production. This treatment was considered as necessary due to the environmental conditions in the greenhouse. It is important to slow down the seedling growth in order to more appropriately score the seedling infection type.

One-week old seedlings were inoculated with the single isolates by blowing over the plants 0.1 g/tray of a mixture of talcum and spores (6 to 1 ratio; v/v). After inoculation, seedlings were incubated separately in darkness for 24 h at 18°C and 100% relative humidity. Then trays were transferred to the greenhouse in separate transparent plastic chambers and maintained at a temperature ranging from 20–22°C (day) to 16–18°C (night) with 16 h daylight. Leaf rust infection types (ITs) were recorded after ca. 14–16 days, at the two-leaf stage, once the check cvs. reached the maximum level of infection and the number of urediosori did not increase any further. The ITs were recorded using both the 0–4 scale of Long and Kolmer (1989) and the 0–9 decimal scale of McNeal et al. (1971). While the scale used by Long and Kolmer is effective to describe the plant-pathogen interaction at a qualitative level, the McNeal' scale is more suitable to provide a measure of the sporulation intensity. In particular, IT0 means complete absence of symptoms; IT1 and IT2 minute or small necrotic/chlorotic flecks, respectively, and no sporulation; IT3 pustules surrounded by clear and serious necrosis, many hypersensitive flecks and traces of sporulation; IT4 to IT6 clear chlorosis around the pustules and from traces (IT4) to high level of sporulation with only few flecks without sporulation (IT6); IT7 to IT9 some (IT7) to no chlorosis at all (IT9) with abundant sporulation. IT included between 0 and 3 indicates a resistant response, IT from 4 to 6 refers to intermediate responses showing some degrees of resistance and IT from 7 to 9 indicates susceptibility.

Molecular analysis

A genetic map based on SSR and DArT markers was obtained from the 176 C × L RILs. The SSR marker sets publicly available in GrainGenes (updated to January 2005) were used to search for polymorphisms between the two parents of the mapping population. The SSR probe sets used (http://wheat.pw.usda.gov) were: BARC (*Xbarc* marker loci), CFA (*Xcfa*), CFD (*Xcfd*), CNL, KSUM, WMC (*Xwmc*) and WMS (*Xgwm*).

A unique thermo-cycling protocol was used for all primer sets: initial denaturation at 94°C, 3 min; 20 cycles of touch-down 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 two parents were evaluated in 5% polyacrylamide manual sequencing gels (45 cm long), stained with the silver-nitrate method (Bassam et al. 1991). Depending on the allele base pair differences and the complexity of the marker's profile, selected SSRs were profiled on the entire RIL population using either 3% agarose gel electrophoresis, or the automated LI-COR 4200 IR^2 System (LiCor, Lincoln, NE, USA) with forward primers labeled with IR700/IR800 fluorochromes.

Diversity Arrays Technology (DArT markers) was used to saturate the map. DArT markers are hybridization-based markers obtained from genomic clones genotyped on a microarray platform (Mantovani et al. 2008) developed by Triticarte® Pty. Ltd (Canberra, Australia, http://www.triticarte.com.au) using proprietary hexaploid and tetraploid wheat clones. The prefix "wPt" indicates DArT clones obtained from hexaploid wheat (genomic DNA restricted with the combination of *Pst*I and *Taq*I enzymes) while DArT markers identified by a code including numbers only were obtained from tetraploid wheat (Kilian and Wenzl, unpublished data).

The genetic map (Mantovani et al. 2008) was built based on 554 markers, including 160 SSRs, two EST-SSRs and 392 DArT markers joined into 17 main linkage groups and two small groups including a few markers each. The total length of the map was equal to 2,022 cM.

The map construction was carried out according to the procedure described by Akbari et al. (2006). Linkage groups and the optimal marker order within linkage group were identified using different mapping programs: JoinMap v. 4.0 (Stam 1993; http://www.kyazma.nl/index.php/mc.JoinMap/) and RECORD (Van Os et al. 2005). The final map was obtained using the new mapping program EasyMap v. 0.1, being developed at Diversity Arrays Technology P/L (Wenzl et al., unpublished), that includes the algorithm used by RECORD for optimising marker order. Each linkage group was assigned to the corresponding durum wheat chr. based on the colinearity with the wheat SSR maps (Ta-SSR-2004 and Ta-Composite-2004; Somers et al. 2004; http://wheat.pw.usda.gov/). Based on the Somers' SSR consensus map, we estimate that our map covers ca. 70% of the wheat A and B genomes.

The molecular map used for the QTL analysis was based on 213 markers; in fact, in case of co-segregating markers mapped within a 5 cM interval, only the most informative one was retained.

The SSR markers used to genotype the panel of advanced lines from different crosses and their resistant parents Creso, Colosseo and Plinio were separated in the automated LI-COR 4200 IR^2 System (LiCor, Lincoln, NE, USA). A total of 57 SSRs were profiled, 43 of which were

evenly distributed over the 14 chrs. of the A and B genomes and, being characterised by a high polymorphism content, were useful to estimate the familial relationships among and within the groups (different crosses) of accessions. The remaining 14 SSRs were selected in the chr. 7BL region found to harbour the major leaf rust resistance QTL in the C × L population. The SSR markers and their order were based on the results of a durum wheat join map obtained with JoinMap v. 4.0 using the segregation data of two durum RIL populations. One population including 249 RILs from Kofa × Svevo was described by Maccaferri et al. (2008), and the second was the C × L mapping population described above.

Statistical analysis

Before conducting QTL and association analysis, frequency distributions of the phenotypic data were inspected to assess the consistency of data through visual scoring dates, traits and years and to estimate the complexity of the genetic control of the traits. Box-Cox normality plot (carried out in Minitab®15, Minitab Ltd, Coventry, England) was used for all the phenotypic data-set considered: only the data obtained in 2006 for the advanced lines related to Creso were then subjected to square root transformation before proceeding with the analysis of variance (ANOVA) and association mapping.

ANOVA was carried out separately for each trial and the heritability (h^2) was calculated on a mean basis across three replications according to the following: $h^2 = \sigma^2_G/(\sigma^2_G + \sigma^2_E/r)$. Heritability should be considered as being "narrow sense" because the genetic variance included only the additive component and, possibly, the additive × additive epistatic interaction.

Composite interval mapping (CIM; Zeng 1994) was used to search for QTLs using the infected leaf area index (LRS) and the AUDPC data scored for the C × L RILs in 2006.

CIM analysis was performed in Windows QTL-Cartographer version 2.5 (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm; Wang et al. 2005). The parameter set-up of "model 6 standard analysis" was used: walk speed of 2-cM step, "forward and backward" regression for the selection of the markers to control for the genetic background (control markers or cofactors) and a blocked window size of 10 cM to exclude closely linked control markers at the testing site. Before conducting CIM, the selected background markers were checked individually to prevent tightly linked multiple markers tagging single QTLs from being included in the model.

The most appropriate genome-wide LOD threshold (experiment-wise P value $\leq 5\%$) to be used in the CIM analysis was searched by means of permutation analysis carried out for each trait-environment combination being analysed (10,000 permutations/analysis). In the RIL field trial, the LOD thresholds ($P \leq 0.05$) for LRS measured at three subsequent (early, medium and late) stages of infection development and for AUDPC ranged from 2.96 to 3.34; thus, a general LOD threshold equal to 3.0 was used for all of these traits. A similar LOD threshold range (from 2.70 to 3.11; $P \leq 0.05$) was obtained when a subset of RILs fixed for the susceptibility allele at the major leaf rust resistance QTL was used in a complementary analysis carried out to better investigate for the presence of QTLs with minor effects. Finally, the same LOD threshold (3.0) was used also for the analysis of agronomic traits such as HD, TKW and TW. As to the latter two traits, QTL analysis was performed using the differences between the RIL values measured in the disease-protected field trial and in the inoculated one.

In addition to the biparental mapping analysis, an association mapping approach (Ersoz et al. 2007) was used. The familial relationships among and within the groups of 62 advanced lines related to Creso were estimated based on the molecular data at 43 SSR markers evenly distributed over the 14 wheat chrs. and characterised by null or limited inter-marker linkage disequilibrium (LD) values. A 62 × 62 relationship matrix (K matrix) was obtained using the kinship coefficient in TASSEL v. 2.0.1 (http://www.maizegenetics.net/). Association analysis for the SSR markers at the chr. 7BL region was performed using a mixed linear model (MLM) as implemented in the software TASSEL (Yu et al. 2006). Briefly, the model herein used includes the genetic markers as fixed effects and the polygene background effect as random effect, the latest being estimated through the K matrix of relative kinship coefficients that define the degree of genetic covariance between pairs of individuals. Only alleles with a frequency higher than 10%, i.e. more than 6 counts, in the advanced line set (hereafter indicated as frequent alleles) were considered in the association test.

The LD estimates (D' and P values) for each pair of markers (SSRs with multiple alleles) mapping in the 7BL distal chr. region were calculated as in Farnir et al. (2000), according to the equation for markers with multiple alleles using the software TASSEL; LD P values were calculated using 10,000 permutations.

Results

Major and minor QTLs for leaf rust resistance

Leaf rust infection response was recorded from two sets of genetic materials related to the durum wheat cv. Creso (i.e. the source of durable leaf rust resistance): a set of RILs

and a set of advanced lines from diverse crosses involving Creso or its resistant derivatives as parental genotypes.

Considering the results of the RIL population tested in a replicated field trial with artificial inoculation (2006), it is interesting to note that the frequency distributions for LRS at three different dates and AUDPC (Fig. 1) indicated the presence of a major gene/QTL accounting for most of the phenotypic variation. The heritability coefficients of the traits were quite high reaching values equal to 72, 82 and 82% for LRS at the early, medium and late stages of infection development, respectively, and equal to 83% for the AUDPC values. The susceptible parent Lloyd confirmed its response to the pathogen, and showed increasing values for LRS during the infection development, up to 46.9% in the latest stage considered, and an AUDPC equal to 297.6 units, while Colosseo showed a substantially resistant response throughout the disease cycle. As Fig. 1 shows, the presence of a number of RILs with a susceptible response to leaf rust infection classified as intermediate between those of the two parents and the absence of a clear bi-modal phenotypic distribution suggested that some additional resistance genes were segregating in the population in addition to the major gene contributed by Colosseo. HD, TKW and TW also showed high heritability values (>80%, data not reported) in both the inoculated and disease-protected trials.

The QTL analysis carried out using the disease response data (three LRS scorings and AUDPC) of all the RILs allowed us to uncover only one major QTL. This major QTL (*QLr.ubo-7B.2*), with the allele for leaf rust resistance contributed by Colosseo, was mapped in the distal region of the chr. 7BL arm (wheat deletion bin 7BL10-0.78-1.00), within a 5 cM interval (LOD—2 supporting interval) flanked by microsatellite markers *Xbarc340.2* and *Xgwm146* in the upper part and by *Xgwm344.2* in the distal part of the region (Table 1, upper section). The QTL was detectable across the complete cycle of leaf rust infection development, with very high LOD values for all the LRS scorings (Fig. 2) as well as for AUDPC. R^2 values for the LRS ranged from 49.8% of the phenotypic variance at the early stage of disease development (i.e. kernel milk stage of the plant, Zadoks 75) to 76.9% in the late phase (i.e. at the end of the grain-filling period, Zadoks 80). For AUDPC also a very high R^2 value (72.9%) was detected. The additive effects (computed as half of the phenotypic difference between the means of the two RIL groups homozygous for the Lloyd and the Colosseo allele, respectively, at the QTL peak position) ranged from 6.6 to 18.4% of LRS in the early onset and in the late stage of the disease cycle, respectively. The effect of the QTL was also large on both kernel weight and test weight, with a gain equal to 1.8 g per 1,000 kernels and 0.8 kg hl^{-1} in favor of the RILs carrying the resistance allele from Colosseo, respectively, as it resulted from the QTL analysis of the differences between the values measured in the disease-protected trial and in the artificially inoculated one (Table 1, upper section).

Although a total of four QTLs were detected for HD, none of them was located in the chr. region harbouring *QLr.ubo-7B.2* (data not shown).

The presence of additional minor QTLs segregating in the population was investigated by means of selective CIM analysis carried out on a subset of lines (76 RILs in total) with the molecular haplotype homogeneous to Lloyd at the *QLr.ubo-7B.2* significance (LOD 3.0) chr. interval. Permutation analysis (experiment-wise P value ≤ 0.05) indicated that a LOD threshold equal to 3.0 was adequate to analyse this subset of lines for all the considered traits. This analysis allowed us to identify three additional QTLs with a significant effect on leaf rust resistance (Table 1, lower section). For two of the three QTLs, the resistance allele was contributed by Lloyd (*QLr.ubo-2A* in the distal

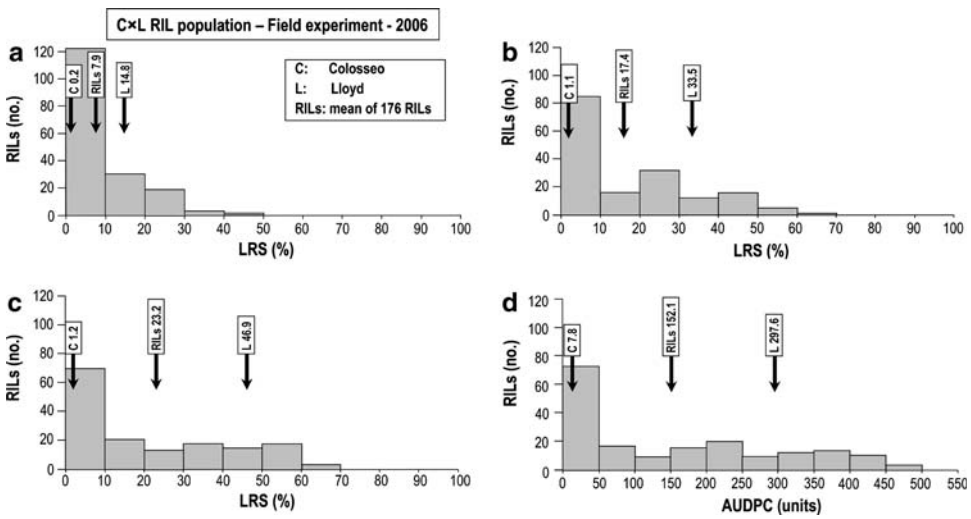

Fig. 1 Phenotypic distributions for the infected leaf area (measured as leaf rust susceptibility index, LRS) and area under disease progress curve (AUDPC) in the 176 Colosseo × Lloyd RILs. Means for RILs (RILs), Colosseo (C) and Lloyd (L) are indicated with *arrows*. The figure shows the distribution of the RILs in the field trial carried out in Argelato during 2006. The distributions for the LRS at the early (**a**), medium (**b**) and late (**c**) stages of the disease developmental cycle and of AUDPC (**d**) are reported

Table 1 QTLs identified for leaf rust susceptibility index (LRS, i.e. the percentage of infected leaf area) recorded at three different stages (early, medium and late) of the disease cycle and area under progressive disease curve (AUDPC) in the Colosseo × Lloyd RIL population tested in the field under artificial inoculation in 2006. For each QTL, the LOD peak, its chromosome location, the determination coefficient (R^2) and the (LOD−2) supporting interval are reported as from the composite interval mapping analysis

QTL and flanking markers	Trait (RILs–2006)	LOD peak (LOD units)	Peak position (cM)	R^2 (%)	Additive effect[a] (Trait units)	(LOD−2) supp. int. (cM)
QTL analysis carried out on the complete set of 176 RILs						
QLr.ubo-7B.2 (*Xbarc340.2/312780*)	LRS-early (%)	28.6	214	49.8	6.6	209–218
	LRS-medium (%)	44.7	214	67.9	14.5	207–218
	LRS-late (%)	47.9	211	76.9	18.4	207–214
	AUDPC (units)	44.5	209	72.9	126.5	207–218
	TKWΔ[b] (g)	3.0	212	20.2	0.9	204–218
	TWΔ[c] (Kg/hl)	4.1	212	16.3	0.4	201–218
QTL analysis carried out on the subset of 76 RILs with the susceptible haplotype at QLr.ubo–7B.2						
QLr.ubo–2A (*wPt-386/310911*)	LRS-early (%)	6.8	67	30.0	−4.8	58–80
	LRS-medium (%)	5.2	74	18.6	−5.6	62–80
	LRS-late (%)	–	–	–	–	–
	AUDPC (units)	4.2	72	19.1	−45.8	58–80
QLr.ubo-3A (*311707/Xwmc664*)	LRS-early (%)	–	–	–	–	–
	LRS-medium (%)	4.1	56	24.8	−6.4	32–70
	LRS-late (%)	–	–	–	–	–
	AUDPC (units)	4.3	54	31.1	−57.9	29–68
QLr.ubo-7B.1(*Xwmc405.1/Xgwm573*)	LRS-early (%)	4.6	59	15.9	3.7	45–66
	LRS-medium (%)	3.0	59	9.7	4.1	45–66
	LRS-late (%)	4.2	58	19.4	5.8	45–66
	AUDPC (units)	4.1	58	14.2	39.7	45–66

The major QTL for leaf rust resistance (*QLr.ubo-7B.2*, with the resistant allele inherited from the parental cv. Colosseo) was detected with the complete set of 176 RILs, while the three additional QTLs were detected using a subset of 76 RILs with the *QLr.ubo-7B.2* susceptible haplotype (inherited from the parental cv. Lloyd). The effects of *QLr.ubo-7B.2* on thousand kernel weight (*TKW*) and test weight (*TW*) are also reported

[a] The additive effect is reported as half of the phenotypic difference between the two groups of RILs homozygous for the allele inherited from the parental cv. Lloyd (susceptible parent) and Colosseo (resistant parent), respectively

[b] TKWΔ: difference between the thousand kernel weight measured in the leaf rust inoculated field trial and in the protected one

[c] TWΔ: difference between the test weight measured in the leaf rust inoculated field trial and in the protected one

region of chr. arm 2AL and *QLr.ubo-3A* in the proximal region of chr. 3AS); both QTLs were significant for AUDPC and for LRS in the early and/or medium disease development phases, but not in the late phase. The additive effect at both QTLs was equal to ca. 5% for the infected leaf area and ca. 50 units for AUDPC. A third QTL (*QLr.ubo-7B.1*) was mapped on the proximal region of chr. 7BS, with a significant effect across the entire cycle of disease development but with an overall rather limited effect (from 3.7 to 5.8% for LRS and 40 units for AUDPC); in this case the resistance allele was contributed by Colosseo.

The density of markers mapped in the *QLr.ubo-7B.2* region was high: some 29 markers (1 EST-SSR, 5 SSRs and 23 DArTs) were mapped in the distal region of chr. 7BL within a 50 cM-wide interval. Table 2 reports the results of a simple regression analysis of LRS at the late stage of disease development and AUDPC data on the two genotypic classes at markers located in this QTL region. It is possible to observe a smooth increase of the regression significance (LOD and *P* values) along the region, with a more relevant increase from the region tagged by *Xbarc340.2* and *Xgwm146*, up to the peak of significance close to the distal end of the chr.; a slight decrease in significance was observed at the end of the linkage group.

Effects of *QLr.ubo-7B.2* on leaf rust resistance in a panel of lines related to Creso

In a parallel study, the effects of *QLr.ubo-7B.2* were investigated in an association-based study carried out on a panel of 62 advanced lines related by various pedigree relationships to Creso or its leaf rust resistant derivatives Colosseo and Plinio. Two inoculated field trials were carried out in 2006 and in 2007. The second year was characterised by a more intense disease pressure; in fact,

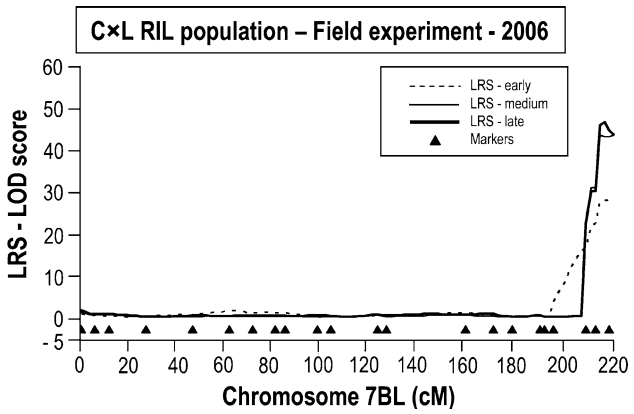

Fig. 2 Composite interval mapping results for the 176 Colosseo × Lloyd RILs assessed for leaf rust resistance in Argelato during 2006. The LOD profiles of chr. 7B, harbouring the major resistance QTL, are shown for the percentage of infected leaf area (LRS), at the early (*dashed line*), medium (*solid*, *thin line*) and late (*solid*, *thick line*) stages of the leaf rust developmental cycle in the field. The genetic position of markers along the chromosome used in the QTL analysis is indicated using *solid triangles*

the mean LRS values of the advanced lines at late stage of the disease cycle were equal to 14.0 and 48.1%, with ranges from 0 to ca. 60% and from 0 to ca. 80%, in 2006 and 2007, respectively.

It is interesting to underline that for all the response traits (LRS at different stages and AUDPC) a wide range of variation was detected in both years, with several lines showing LRS percentages and AUDPC values significantly lower or higher than Creso and it derivatives, especially in the second year, thus suggesting the presence of additional leaf rust resistance-susceptibility factors in the panel tested. The detailed data for each advanced line, leaf rust resistance donors and susceptible checks recorded in 2006 and 2007 years are reported as supplementary materials. In 2006, the h^2 values for LRS at the early and late stages of infection development were equal to 49 and 66%, while in 2007 the values ranged from 78% in the early stage to 91% in the late stage; AUDPC heritability values were equal to 63% in 2006 and 88% in 2007.

Table 2 Results of the single marker analysis (simple linear regression model) based on the 176 Colosseo × Lloyd RIL field response data for all the SSR and DArT markers mapped in the chr. 7BL region harbouring the major QTL for leaf rust resistance (*QLr.ubo-7B.2*)

Markers	Chr. position	LRS-late (%)			AUDPC (units)		
	(cM)	*P*	LOD	*b*	*P*	LOD	*b*
TC69382	167.3	NS	0.50	2.46	NS	0.62	19.06
305954	168.9	NS	0.28	1.85	NS	0.37	15.02
wPt-4814	169.8	NS	0.30	1.92	NS	0.42	16.05
wPt-0194	174.4	**	1.51	4.37	*	1.71	32.28
wPt-2356	183.4	***	2.94	5.67	***	2.93	41.28
wPt-3086, wPt-4300	184.3	****	3.79	6.33	****	3.76	46.67
Xgwm577	187.8	****	3.88	6.36	****	3.81	46.75
wPt-0217, wPt-6104, 378041	189.8	****	4.29	6.72	****	4.25	50.03
wPt-3939	190.8	****	3.75	6.68	****	3.84	50.49
Xgwm783	193.3	****	4.18	7.24	****	4.12	50.50
Xwmc276	196.5	****	4.90	7.66	****	4.56	51.94
Xbarc340.2	209.8	****	23.11	14.37	****	20.95	96.63
Xgwm146	212.7	****	30.22	15.71	****	28.09	107.39
372675, wPt-0504	215.4	****	45.26	17.54	****	41.30	120.19
wPt-6869, wPt-7219	215.7	****	46.79	17.69	****	43.01	121.42
Xgwm344.2	217.2	****	49.64	17.96	****	45.34	123.13
wPt-4038, wPt-1085	218.5	****	50.23	18.06	****	45.55	123.72
378059	218.8	****	41.66	18.11	****	38.43	117.65
wPt-4259	219.1	****	49.64	18.04	****	45.34	123.13
304264, 312227, 312780	220.0	****	49.41	17.17	****	44.72	122.89
304724	221.4	****	34.07	16.22	****	30.85	110.97

The *P* values are based on the *F* test, the LOD scores are based on the likelihood ratio test and the *b* coefficients are the slope coefficients of the linear regression. Results are reported for the leaf rust susceptibility index (LRS, i.e. the percentage of infected leaf area) recorded at the late stage of the disease cycle and for the area under progressive disease curve (AUDPC)

Significance level: *NS* not significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; **** $P \leq 0.0001$

Following the results of the Box-Cox normality plot, the data collected in 2006 were square-root transformed prior to performing the marker-phenotype association analysis.

A series of 14 genomic SSR loci spanning the distal portion of chr. 7BL (including the region harbouring *QLr.ubo-7B.2*) were considered for assessing the association of the allelic variants *vs.* LRS and AUDPC values (Fig. 3). In this respect, it is worth noting that the parental cvs. Colosseo and Plinio (resistant derivatives of Creso) showed a molecular haplotype homogeneous to Creso at the chr. 7BL region.

In the advanced lines, the LD values detected for pairwise comparisons among the set of loci mapping in the 7BL region (Fig. 3) revealed an LD pattern similar to the one that could be expected in a bi-parental RIL population, with highly significant *P* values and high D' values (>0.30–0.50) over distances of 10 or more cM. Thus, the number of markers herein used seems adequate to validate the results obtained with the C × L RIL population.

The results of the association analysis in both years agreed with those obtained from the C × L mapping population. In fact, out of the 14 SSRs spanning the 7BL distal chr. region (Fig. 3), only five (*Xwmc526.1*, *Xwmc526.2*, *Xbarc340.2*, *Xgwm146* and *Xgwm344.2*), located within the *QLr.ubo-7B.2* significance interval (as detected in the RIL mapping population, LOD threshold equal to 3.0), were found to be significantly associated ($P \leq 0.001$) to LRS at the late stage in both years (Table 3). Further, *Xgwm146* and *Xgwm344.2* were associated also to AUDPC and showed the highest significance level among the markers mapping in the *QLr.ubo-7B.2* region. Marker *Xcfa2257.2*, located near *Xgwm344.2* in the QTL region (see Fig. 3), could not be considered for the association analysis due to the high frequency of null alleles, including the resistant parents Creso and its derivatives.

All the other markers, located in the upper part of the distal chr. 7BL region outside of the QTL significance interval (see Fig. 3), did not show a significant association to leaf rust infection response, except for *Xgwm577* which was the only SSR mapping outside the major QTL significance interval showing a differential allelic response ($P \leq 0.05$) when considering the LRS in the early stage in 2006 (data not reported). *Xgwm783* and *Xbarc182* (located outside the QTL region) were not considered for the association test due to difficulties in distinguishing the banding patterns of the lines.

Table 4 reports the average phenotypic value and the number of lines for each frequent allele present at the five SSR markers (*Xgwm526.1*, *Xgwm526.2*, *Xbarc340.2*,

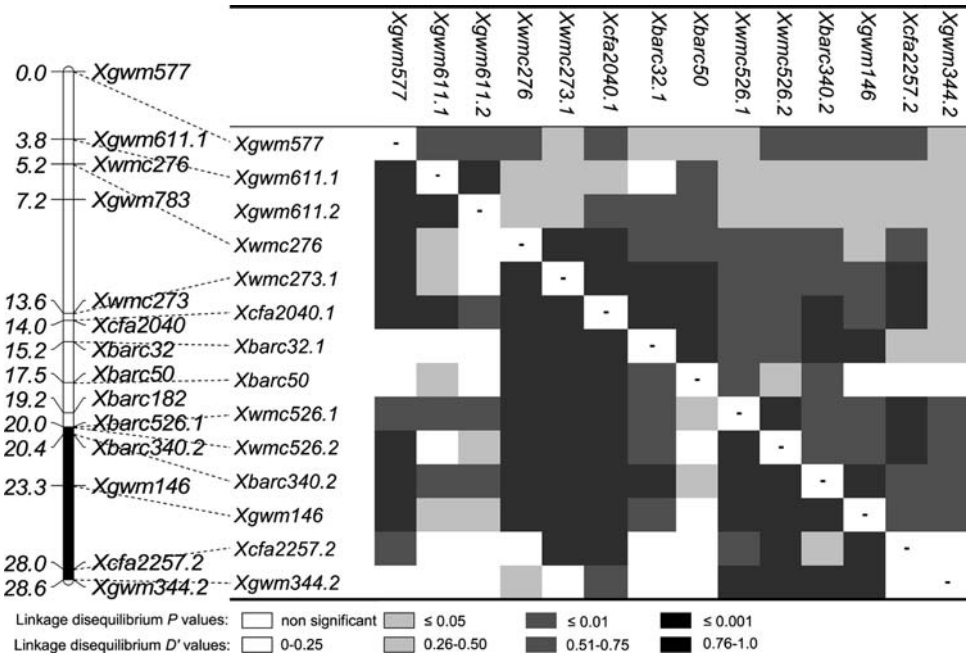

Fig. 3 SSR-based durum wheat genetic map of the distal chromosome 7BL region harbouring the major QTL for leaf rust resistance. The local linkage map is based on the joint analysis of segregation data from two RIL populations obtained from Colosseo × Lloyd and Kofa × Svevo crosses. The chr. region harbouring *QLr.ubo-7B.2* is indicated by the solid portion of the chr. bar. Linkage disequilibrium (LD) values and LD-probability estimates among SSR markers are calculated using the panel of 62 advanced lines (from 21 diverse crosses) related to Creso. LD D' measures are reported above the diagonal line of the table, while LD probability estimates are reported below the *diagonal line*. The chr. position of *Xgwm611.2* was assumed as in Peng et al. (2000), while *Xgwm783* and *Xbarc182* were not genotyped in the set of advanced lines due to their poor electrophoretic profiles

Table 3 Probability values (P) and determination coefficients (R^2) of the marker-phenotype association test (mixed linear model) based on the 62 advanced lines related to Creso and obtained by contrasting the allele inherited from Creso (or from its resistant derivatives) against the pool of all the other alleles at each of the SSR markers mapped in the chr. 7BL region harboring *QLr.ubo-7B.2* (as in Fig. 3)

Markers	Position	Alleles	LRS—late				AUDPC			
			2006		2007		2006		2007	
	(cM)[a]	(no.)	P	R^2	P	R^2	P	R^2	P	R^2
Xwmc526.1	20.0	2	***	15.8	NS	–	***	16.0	NS	–
Xwmc526.2	20.0	2	***	16.2	NS	–	***	17.5	NS	–
Xbarc340.2	20.4	2	***	17.9	NS	–	***	16.2	*	4.1
Xgwm146	23.3	2	***	36.6	*	6.3	***	35.9	*	6.7
Xgwm344.2	28.0	2	***	29.2	**	8.2	***	28.4	**	10.4

Results are reported only for the SSRs showing significant associations with the leaf rust susceptibility index (LRS, i.e. the percentage of infected leaf area) at the late stage of the disease cycle and the area under progressive disease curve (AUDPC) measured under field conditions and artificial inoculations in 2006 and 2007. The test accounts for population structure effects due to familial relationships among lines

Significance level: *NS* not significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

[a] Cumulative genetic distances (from marker *Xgwm577*) as estimated from an integrated map obtained from two durum (Colosseo × Lloyd and Kofa × Svevo) RIL populations

Table 4 Mean leaf rust response phenotypic values associated to the frequent (i.e. with a frequency higher than 10%) alleles present in the panel of 62 advanced lines related to Creso at the SSRs significantly associated to leaf rust response in the 7BL distal chr. region

Marker	Allele (bp)[a]	Allele frequency (no.)	LRS-late 2006 (%)	AUDPC 2006 (units)	LRS-late 2007 (%)	AUDPC 2007 (units)
Xwmc526.1	152	14	17.9	48.2	50.9	251.7
	150	11	21.8	59.9	55.4	286.2
	148	29	7.5	20.4	46.1	232.4
	146	8	19.7	51.8	40.6	208.8
Xwmc526.2	176	8	14.3	37.4	42.4	187.5
	170	34	8.1	22.0	46.5	234.2
	168	12	20.4	54.5	47.0	245.3
	166	8	28.8	79.6	62.5	334.4
Xbarc340.2	Null	21	17.0	45.5	43.2	216.2
	231	26	4.7	11.0	42.1	205.8
	230	14	26.2	75.6	65.3	345.1
Xgwm146	178	20	25.9	73.6	58.9	316.1
	170	29	4.4	10.4	41.7	203.9
	166	11	14.5	38.2	44.6	210.0
Xgwm344.2	Null	11	28.1	79.0	59.4	314.0
	120	34	7.1	17.9	41.9	200.2
	118	11	25.8	71.7	50.9	273.9

The mean values of leaf rust susceptibility index (LRS, i.e. the percentage of infected leaf area) at the late stage of the disease cycle and of the area under progressive disease curve (AUDPC) measured in the field under artificial inoculation in 2006 and 2007 are reported

[a] For each SSR, the allele identical by descent to Creso is highlighted in bold

Xgwm146 and *Xgwm344.2*) significantly associated to leaf rust response. Consistently with the presence of the major QTL for leaf rust resistance, the alleles most probably inherited either directly from Creso or indirectly through Colosseo or Plinio at these markers were associated to a low leaf rust infection level.

Based on the haplotype composition at the five SSRs mapped in the chr. 7BL region harboring *QLr.ubo-7B.2*, the advanced lines were assigned to three groups: group *a*, including 20 lines derived from 11 different crosses with the complete haplotype (spanning 8.6 cM) homogeneous to Creso, Colosseo and Plinio; group *b*, including 22 lines with haplotype rearrangements within the region; and group *c*, comprising 20 lines with haplotypes unrelated to Creso. For sake of conciseness, the haplotype composition at the *QLr.ubo-7B.2* region and the corresponding

phenotypic values of each advanced line are shown in supplementary materials and herein only the comparison among groups will be discussed. In the MLM-based association test, highly significant differences in the leaf rust response were observed between the set of lines carrying the haplotype homogeneous to Creso vs. the two sets of lines with either rearranged haplotypes or completely unrelated haplotypes. The lines of the group *a* showed, on average, a 2.9 and a 36.3% LRS at the late infection stage in 2006 and 2007, respectively, while the lines belonging to groups *b* and *c* showed mean LRS values equal to 12.6 and 26.5% in 2006, and to 54.6 and 52.8% in 2007, respectively. Mean AUDPC values for the set of lines homogeneous to Creso were equal to 6.7 in 2006 and 174.6 in 2007; these values are consistent with those recorded for Creso and its derivatives and consistently lower than the values observed for the lines with the rearranged and unrelated haplotypes (AUDPC of the latter group equal to 73.1 in 2006 and 279.5 in 2007), as well as for the susceptible checks included in the field trials.

A few advanced lines included in group *a* (lines 2/1, 2/3, 20/3 and 20/10) showed a leaf rust response more susceptible than Creso, Colosseo and Plinio, particularly in 2007 under very high disease pressure. On the contrary, lines with a leaf rust resistance similar to or more higher than that showed by the resistance donor parents were observed in group *b* and, to a lesser extent, in group *c*, consistently with the presence of other leaf rust resistance genes segregating in these materials.

Leaf rust response of the Creso-derived resistance at the seedling stage

In preliminary experiments, the leaf rust response of some genotypes, including Colosseo and Lloyd (resistant and suceptible parent of the RIL population, respectively), the resistant cv. Creso, the suceptible cv. Aconchi and UCRD05-2 (an accession carrying *Lr19* in the Aconchi background), was tested at the seedling stage with each one of the 16 isolates that were used in bulk to artificially inoculate the field trials. Resistance of Creso and its derivative Colosseo to the majority of the leaf rust isolates varied from complete to moderate, with a clear hypersensitive infection type (IT). However, it should be underlined that two out of the 16 isolates evidenced a clear virulence pattern on the above two resistant cvs. (data not reported). The two cvs. Lloyd and Aconchi confirmed a susceptible response to all the isolates, while UCRD05-2, carrying the translocation Ag 1-23 with the *Lr19* gene, showed a completely immune response (IT = 0 on the McNeal' scale), clearly different from that shown by Creso and its derivatives.

In order to investigate the possible role of the chr. region harboring the major QTL (*QLr.ubo-7B.2*) for the adult plant field resistance in controlling the seedling IT response, four isolates, representative of the range in virulence found in the preliminary experiment, were chosen to carry out further seedling tests on 36 RILs selected on the basis of their DArT and SSR haplotype at the chr. 7BL region (see Table 2 for the detailed list of the markers used). Ten RILs were completely homogeneous to Colosseo and ten to Lloyd, while the remaining 16 RILs showed recombination events in the target region. Seedling IT responses (average and range of variation) of the tested materials are reported in Table 5. Analysis of variance showed highly significant differences among genotypes when inoculated with isolates 1, 9 and 13 ($P \leq 0.001$), while no significance was detected with isolate 16; in this case, all genotypes were characterised by a susceptible response. In particular, Colosseo showed the following responses: resistant, though not immune, vs. the isolates 1 and 13 (mean IT values $\leq$3.0), compatible medium-resistant and completely susceptible *vs*. isolates 9 and 16, respectively (mean IT values = 5.3 and 8.7, respectively), while Lloyd showed a susceptible response to all isolates, with mean IT values ranging from 7.0 to 8.0. A figure showing the different reactions of the two RIL parents is presented as supplementary materials.

The alleles at the *QLr.ubo-7B.2* region did not differentially affect the seedling reaction following inoculation with the isolate 16 virulent to Colosseo (see RIL group mean values in Table 5). On the contrary, when inoculated with the three isolates (1, 9 and 13) avirulent to the genetic factor carried by Colosseo, the two groups of RILs with a contrasting haplotype at the *QLr.ubo-7B.2* (homogeneous to Colosseo and Lloyd, respectively) where characterised, on average, by a different ($P \leq 0.01$) IT response with the Colosseo allele conferring leaf rust resistance. Moreover, the mean IT values of the 7BL-recombinant RILs were within those of the two above-mentioned groups, with single RIL values ranging from very low (2) to very high (9). The presence at the *QLr.ubo-7B.2* region of a resistance effect at seedling stage was supported by the results of the single marker linear regression carried out using the IT scores recorded for each of these three isolates on the group of 16 RILs with recombinant haplotypes at the QTL region (Table 6). For all the three isolates, the marker linear regression pointed out maximum associations at the markers near *Xgwm344.2*.

The seedling IT response of the 36 RILs was correlated with the open field, adult plant response data as recorded in the field experiment carried out in 2006. Correlation coefficients between IT values obtained with isolates 1, 9 and 13 and the LRS and AUDPC values were always highly significant ($P \leq 0.001$), with *r* values ranging from 0.72 to 0.85 (data not reported). No significant association was evidenced between seedling response following

Table 5 Seedling response of Colosseo, Lloyd, the parents of the RIL mapping population, and of three groups of RILs selected on the basis of their haplotype composition at the chr. 7BL region harbouring the major QTL for field response to leaf rust

Genotype	Infection type							
	Isolate 1		Isolate 9		Isolate 13		Isolate 16	
	Range[a]	Mean	Range	Mean	Range	Mean	Range	Mean
Colosseo	3–3	3.0	5–6	5.3	2–3	2.7	8–9	8.7
Lloyd	6–8	7.0	6–8	7.0	8–8	8.0	7–8	7.3
10 RILs with "Colosseo" haplotype	3–4/4–6	4.8[ab]	3–4/5–6	5.5 a	3–3/4–5	4.5[a]	6–8/9–9	7.9[a]
10 RILs with "Lloyd" haplotype	6–8/7–9	8.1[b]	8–9/9–9	8.6 b	7–9/9–9	8.6[b]	6–8/8–9	7.9[a]
16 RILs with "recombinant" haplotype	2–2/7–8	5.8[c]	3–3/9–9	6.3 c	2–3/9–9	5.8[c]	5–7/9–9	7.8[a]
CV (%)[c]	10.2		10.2		11.3		10.2	

The genotypes were tested with four Italian isolates of *Puccinia triticina* Eriks. Infection types (IT) were recorded using the decimal 0-9 McNeal' scale (McNeal et al. 1971)

[a] For Colosseo and Lloyd, the highest and the lowest values of the three replicates are reported; for each of the three RIL groups, the two reported ranges of variation refer to the RIL with the lowest (before slash) and the highest mean IT value, respectively

[b] Values followed by the same letter are not significantly different (LSD $P \leq 0.01$)

[c] Coefficient of variation over the complete set of tested genotypes

Table 6 Results of the single-marker association test (linear regression model) based on the seedling IT responses of 16 Colosseo × Lloyd RILs showing recombination events in the chr. 7BL region harbouring the major QTL for field response to leaf rust

Markers	Chr. position (cM)	Infection type response								
		Isolate 1			Isolate 9			Isolate 13		
		P	LOD	*b*	*P*	LOD	*b*	*P*	LOD	*b*
372675, wPt-0504	215.4	**	2.21	1.55	**	2.56	1.42	***	3.12	1.81
wPt-6869, wPt-7219	215.7	****	4.34	1.92	****	5.11	1.74	****	6.06	2.16
Xgwm344.2	217.2	****	4.34	1.92	****	5.11	1.74	****	6.07	2.16
wPt-4038, wPt-1085	218.5	****	4.34	1.92	****	5.11	1.74	****	6.06	2.16
378059	218.8	****	4.98	2.02	****	5.54	1.80	****	6.93	2.24
wPt-4259	219.1	****	5.74	2.10	****	5.81	1.83	****	7.80	2.31
304264, 312227, 312780	220.0	****	6.04	2.05	****	4.93	1.71	****	6.91	2.19
304724	221.4	****	6.17	2.07	****	4.97	1.72	****	7.03	2.21

The seedling IT responses are referred to the three isolates (1, 9 and 13) that showed avirulence to Colosseo. The *P*-values and the LOD scores refer to the *F* test and the likelihood ratio test, respectively. The slopes of the linear regression equation (*b* coefficients) are also reported. Only markers at the chr. 7BL distal region showing a significant marker-trait association is reported

Significance level: ns not significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; **** $P \leq 0.0001$

inoculation with isolate 16 and the adult plant open field response (r values <0.08).

Based on the above-reported results, all the 176 C × L RILs were evaluated at the seedling stage using one of the isolates (i.e. isolate 1) characterised by an avirulent response to Colosseo and Creso. The frequency distribution of the seedling IT values was bimodal with two peaks corresponding to the IT classes of the parents Colosseo and Lloyd (Fig. 4). Heritability (estimated on a mean basis) was equal to 92%. The QTL analysis carried out with seedling IT data pointed out the presence of only one significant (LOD > 3) QTL in the distal region of chr. arm 7BL. This major QTL showed a high LOD value peak (32.8) positioned at 218 cM from the top of the linkage group (between *Xgwm344.2* and 378059), with the (LOD−2) support interval comprised within a 10 cM interval between *Xbarc340.2* and the DArT marker 304724 (for the relative position of these markers see Table 2). The position of the major QTL for seedling resistance is thus nearly coincident with that detected for leaf rust response in the field.

Discussion

In our study, the genetic dissection of the leaf rust resistance carried by cv. Creso and its derivatives was primarily addressed by phenotipically assessing adult plant response of recombinant inbred and advanced breeding lines challenged

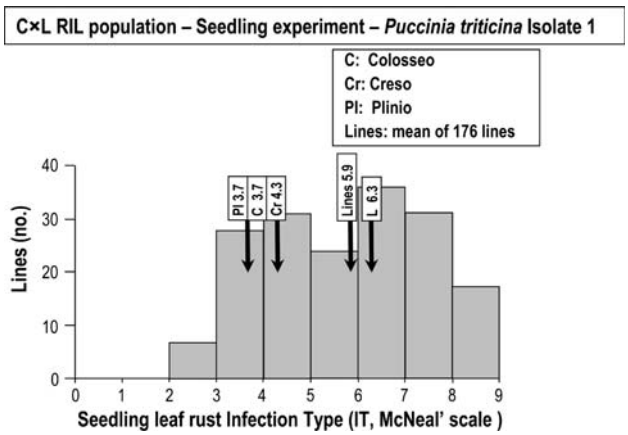

Fig. 4 Phenotypic distribution for seedling infection type (IT) in the 176 Colosseo × Lloyd RILs tested with *Puccinia triticina* Italian isolate 1. Means for advanced lines (*Lines*), Creso (Cr), Colosseo (C), Lloyd (L) and Plinio (Pl) are indicated with *arrows*. Infection type has been recorded following the 0-9 McNeal' scale (McNeal et al. 1971)

with a mixture of isolates under open field conditions. Based on the indications of Martinez and Rubiales (2002) who suggested that the Creso durable resistance was due to a single dominant gene conferring hypersensitive resistance plus additional factors conferring partial resistance, a quantitative genetic approach (QTL analysis) was undertaken to identify and map the genetic determinants of the trait of interest. A mixture of durum wheat rust isolates was used as inoculum in the field experiments to ensure that the prevalent isolates present in the main Italian durum wheat-growing areas were represented. Similar procedures have been widely applied to characterize and map the genetic bases of rust resistance in hexaploid wheat under open field, adult plant conditions (Kolmer and Liu 2002; Schnurbusch et al. 2004; Lillemo et al. 2008).

Our study has ascertained that the durable leaf rust resistance carried by the durum wheat cv. Creso and some of its derivatives is controlled by a major QTL positioned in the distal region of chrom. 7BL and characterised by a high R^2 value and a narrow support interval. In this study, the cv. Colosseo (Mexa's mutant/Creso) was used to develop the RIL population which enabled us to map the major resistance QTL inherited from Creso.

In particular, the segregation data from a Colosseo × Lloyd mapping population allowed us to point out that the leaf rust resistance inherited from Creso is expressed at both seedling (as evaluated under greenhouse conditions using different Italian *Puccinia triticina* isolates) and adult plant (under open field conditions with plants artificially inoculated with a mixture of 16 Italian isolates). The same major QTL is most probably involved in the control of the seedling and the adult plant reactions, as suggested by the nearly complete overlapping between the significance intervals of the unique major QTL underlying a large amount of the phenotypic variation observed for both type of reactions and by the coincidence of the QTL peaks. However, the mapping resolution of the materials herein used does not allow us to discriminate whether the QTL effects are due to a single gene controlling both reactions or to tightly linked factors (located within a range of maximum 10 cM).

Overall, our results contributed to a better knowledge of the phenotypic response to leaf rust carried by Creso. In this respect, the presence of a major dominant factor controlling a large portion of the phenotypic variability had already been reported by Martinez and Rubiales (2002) and more recently by Amaro et al. (2007), based on the inspection of the segregation ratio in F_2 populations and corresponding F_3 progenies obtained from crosses between Creso and susceptible cvs. such as Pedroso (Martinez and Rubiales 2002) and the CIMMYT cv. Atil C2000 (Amaro et al. 2007). These studies were carried out under controlled and open field conditions. Recently, Martinez et al. (2007) have ascertained that several *Puccinia triticina* pathotypes show the same virulence pattern when inoculated at the seedling stage on both Creso and Colosseo and that this pattern is distinct from those on other durum and bread cvs. known for carrying at least one gene for the hypersensitive response. Most interestingly, the large majority of the tested durum wheat resistance donors showed a specific reaction pattern different from those of the known *Lr* genes characterised in hexaploid wheat.

Our observations and those reported by Martinez et al. (2007) concerning the identification of a few isolates characterised by high virulence to Creso and its derivatives support the hypothesis that the race-specific hypersensitive reaction is actually an important feature of the Creso resistance.

Mapping of leaf rust resistance QTLs/genes is relevant to durum breeding since specific studies in durum wheat are still limited (Herrera-Foessel et al. 2007, 2008; Martinez et al. 2007). Our results indicate that the major QTL for Creso leaf rust resistance is located in the deletion bin 7BL10-0.78-1.00 (Sourdille et al. 2004), which is one of the regions of the wheat chr. group 7 with the highest density of EST loci (Hossain et al. 2004). The distal region near the telomeric end of wheat chr. group 7 is known to be rich in resistance genes, resistance gene analogs (RGAs) and defense-response genes (Dilbirligi et al. 2004).

The wheat wild relative *Lophopyrum ponticum* is the donor of *Lr19*, an effective leaf rust resistance gene located in the distal portion of chr. 7EL (Sharma and Knott 1966), which has been introgressed into the corresponding 7D and 7A homoelogous chr. regions of hexaploid and durum wheat, respectively (Zhang et al. 2005; Gupta et al. 2006). Even if *Lr19* appears to map in a homeologous chr. region very close to that identified in this study, the resolution of

the mapping studies carried out so far precludes any useful indication about the possible allelism between *Lr19* and *QLr.ubo-7B.2*.

The two genetically characterised and closely linked genes for leaf rust resistance *Lr14a* (one of the few designated resistance genes which originated from *Triticum turgidum*; Singh et al. 2005) and *Lr14b* have been mapped in the distal portion of chr. 7BL (near *Pm5*, *Ep-B1* and *Wsp-B1* genes, Dyck and Samborski 1970; for the detailed gene map see GrainGenes database, http://wheat.pw.usda.gov/GG2/index.shtml). Recently, the mapping position of *Lr14a* has been more precisely refined using a population of 98 F_3 lines from the Chilean durum Llareta-INIA (Herrera-Foessel et al. 2008): *Lr14/LrLla* mapped very close to markers *Xgwm146* and *Xgwm344.2*. The same two SSRs were identified in our study as the markers most tightly linked to the resistance gene carried by Colosseo.

The comparison between our results and those published by Herrera-Foessel et al. (2005, 2008) shows that the response of Creso and its resistant derivatives is very similar to that of the *Lr14/LrLla* genotypes: both are expressed at seedling and adult plant stages, and both are effective under field conditions of natural leaf rust infection in Mexico and against the BBG/BN Mexican *P. triticina* race (K. Ammar, personal communication; Amaro et al. 2007). On the other side, it is very difficult to compare infection type data obtained from different experiments, plant materials and pathogen isolates. The analysis of the pedigree data failed to provide the precise information required to ascertain or to rule out the presence of identity-by-descent relationships between Creso and Llareta that could account for a common origin of the corresponding resistance genes. In fact, most of pedigree data are generally elusive; in particular, common genetic relationships between the two cvs. could actually be traced back to ancient parents originating from the durum CIMMYT program. In fact, Jori C69, a parent of LLareta, shares with Creso a common origin (mainly Tehuacan 60 and Cappelli); however, the involvement of Jori C69 as potential resistance donor has been ruled out by Herrera-Foessel et al. (2008) based on the observation of its susceptibility to BBG/BN.

The study of resistance gene postulation in durum wheat is complicated by the absence of a common set of differential near isogenic lines such as the Thatcher isolines that are available in bread wheat. The development of precise genetic stocks (preferably durum near isogenic lines with a common and widely susceptible genetic background) for *Lr14a*, *Lr14b* (Oelke and Kolmer 2005; Ordoñez and Kolmer 2007a, b) and *QLr.ubo-7B.2* could facilitate the comparison of the disease reaction type by gene postulation with multi-isolate seedling tests and, most important, by testing for allelism in a homogeneous genetic background. Additionally, the fine mapping of these genetic determinants should allow for the identification of molecular markers tightly linked to the genes (e.g. within a 0.1–1 cM) and in sufficiently high density to provide molecular evidences (haplotype sharing) on the presence of identity by descent (IBD) between the genes/alleles carried by cvs. harbouring *Lr14a* on one side and Creso and its derivatives on the other side.

Additionally, a QTL (*QLr.osu–7BL*) for slow leaf rusting was mapped (QTL peak near to *Xbarc182*) by Xu et al. (2005) in the same chr. region underlying *QLr.ubo-7B.2*.

Beside the presence in the cv. Creso of a major hypersensitive response based on a single dominant gene, Martinez and Rubiales (2002) observed segregation for long latency period when considering the F_2 plants with a compatible interaction; these findings allowed the authors to hypothesize that additional factors conferring partial resistance are present in Creso and that these factors can play an important role in durable resistance. The identification of an additional QTL for leaf rust resistance carried by Colosseo (*QLr.ubo-7B.1*, chr. arm 7BS) supports this hypothesis. It is noteworthy to mention that Lloyd, while showing a susceptible phenotype across field experiments, also contributed two additional QTLs for leaf rust resistance (*QLr.ubo-2A* and *QLr.ubo-3A*), with a significant effect detectable only in the early to medium stages of the disease cycle.

The identification and mapping of the major QTL associated to the durable leaf rust resistance carried by Creso, together with the identification of the associated SSR markers, will enhance the selection efficiency in durum wheat breeding programs and will accelerate the release of cvs. with durable resistance through marker-assisted pyramiding of the tagged resistance genes/QTLs most effective against the attacks of wheat fungal pathogens. The exploitation of the rice–wheat synteny and the recently built-up wheat genomic sequence information will help in fine mapping of *QLr.ubo-7B.2* and in the identification of additional markers suitable for MAS.

Our results are relevant for durum wheat breeding because of the wide exploitation of Creso and its derivatives as a preferred source of leaf rust resistance and other valuable traits in many durum wheat breeding programs in the Mediterranean area.

Acknowledgments Research carried out with the financial contribution of the European Union (BIOEXPLOIT project) and of the Emilia-Romagna Region (PRRIITT Project, Misura 3.4-A CEREA-LAB). The authors thank Sandra Stefanelli and Stefano Vecchi for technical assistance throughout the project.

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