

# A major genetic factor for durable leaf rust resistance in durum wheat maps in the distal region of chromosome arm 7BL

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## ABSTRACT

The genetic basis of leaf rust (*Puccinia triticina* Eriks.) resistance was studied using 176 RILs from Colosseo × Lloyd and 62 advanced lines derived from multiple crosses. RILs were tested under field conditions with a mixture of leaf rust isolates and at the seedling stage with single isolates. In the field experiment, the percentage of infected leaf area was evaluated through the disease developmental cycle and the area under disease progress curve (AUDPC) was calculated. A major QTL (*QLr.ubo-7B.2*) for leaf rust resistance at both adult (field conditions) and seedling stages was identified on the distal region of chr. 7BL. In the field, the QTL showed an  $R^2$  of 72.9% and a peak LOD score of 44.5 for AUDPC. The presence of this major QTL was validated by a linkage disequilibrium-based test using field data of advanced lines from multiple crosses. The association results confirmed the QTL location between *Xbarc340.2* and *Xgwm344.2*, with the corresponding AUDPC  $R^2$  values ranging from ca. 10 to ca. 35% depending on the year. *QLr.ubo-7B.2* maps in a gene-dense region (7BL10-0.78-1.00) known to carry several genes/QTLs in wheat and barley for resistance to rusts and other major cereal fungal diseases, including *Lr14a* and *Lr19*, two major candidates for this gene.

## INTRODUCTION

Although leaf rust is a threat to durum wheat (*Triticum turgidum* L. var. *durum*), genetic and molecular mapping studies aimed at characterizing leaf rust resistance genes in durum wheat (Herrera-Foessel et al., 2005, 2007a; Martinez et al., 2007) have been undertaken only recently. Obtaining cultivars (cvs.) with durable resistance (see Johnson, 1984) is a major target in wheat breeding. Thus, we targeted the genetics of the resistance to *Puccinia triticina* conferred by the durum wheat cv. Creso (released in Italy in 1974 and derived from CIMMYT's and Italian materials). Creso represents an important source of durum wheat durable resistance 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). The objectives of this study were: (i) to map the genetic determinants of the durable resistance to leaf rust from the cv. Creso and its resistant derivatives, and (ii) to identify SSRs linked to the resistance gene to enhance the efficiency of selection for resistant genotypes.

## MATERIALS AND METHODS

A population of 176 recombinant inbred lines (RILs, F<sub>6,8</sub>) was produced by Società Produttori Sementi Bologna (PSB, Bologna, Italy) from the cross between the Italian cv. Colosseo (C) and the North American cv. Lloyd (L). Pedigree of Colosseo (Mexa's mutant × Creso) indicates a direct lineage with Creso, the main source of leaf rust resistance. Lloyd (Cando × Edmore, NDSU) is a susceptible cv. under field conditions in Italy. A panel of 62 F<sub>6,8</sub> lines from the breeding programs of PSB and Sementi Samoggia Srl (Crevalcore, Italy), with diverse pedigrees involving Creso or its resistant derivatives Colosseo and Plinio, was used to validate the major gene for leaf rust resistance inherited from Creso.

Artificially inoculated field trials (three reps) were set up in Argelato, Bologna (Po Valley), Italy. The C × L RILs were evaluated in 2006, while the panel of advanced lines, together with Creso, Colosseo, Plinio and four susceptible checks, were evaluated in 2006 and 2007. Inoculation was carried out with a mixture of 16 leaf rust isolates collected in Italy. Reaction to leaf rust was recorded by visually estimating the percentage of infected leaf area (infected leaf area index, LRS) according to the modified Cobb scale (Peterson et al., 1948). Two to three visual scores were recorded. The area under the disease progress curve (AUDPC) was then calculated. The seedling leaf rust response of the parents and RILs was evaluated using a selection of isolates and the infection type (IT) responses were evaluated using the decimal 0-9 McNeal' scale (McNeal et al., 1971).

A genetic map based on a total of 554 SSR and DArT markers was obtained. The map (2022 cM) included 17 main linkage groups and two additional small groups. QTL analysis was carried out on a map with 213 selected and evenly spaced markers (inter-marker interval ≥ 5 cM). The panel of diverse advanced lines related to Creso was molecularly characterized with: *i*) a set of 43 highly polymorphic and evenly distributed SSRs (used to evaluate the familial relationships among and within the lineages); *ii*) 14 SSRs selected to map in the 7BL chromosome region found to harbour the major leaf rust resistance QTL in the C × L population.

Composite interval mapping (CIM; Zeng 1994) was used to search for QTLs using LRS and AUDPC values from field data and the seedling IT values scored with a single isolate representative of the isolates virulent to Lloyd and avirulent to Colosseo. CIM analysis was

performed in Windows QTL-Cartographer version 2.5 (<http://statgen.ncsu.edu/qtlcart/WQTLCart.htm>).

Association analysis for the SSR markers at the 7BL chr. region was performed using a mixed linear model (MLM) as implemented in the software TASSEL (<http://www2.maizegenetics.net/>). Only alleles with more than six counts in the advanced line set were considered in the association test.

## RESULTS AND DISCUSSION

### *Major and minor QTLs for leaf rust resistance*

The frequency distribution of the RILs tested under open field in 2006 for LRS and AUDPC indicated the presence of a major gene/QTL accounting for most of the phenotypic variation. The heritability ranged from 72 to 82% for LRS (recorded at three stages) and was equal to 83% for the AUDPC values. Colosseo showed a substantially resistant response throughout the disease cycle, while Lloyd confirmed its susceptible response, with LRS values up to 46.9% in the latest infection stage. Heading date (HD), thousand kernel weight (TKW) and test weight (TW) also showed high heritability values, ranging from 81 to 86%.

The QTL analysis carried out using all the RILs evidenced the presence of only one QTL. This major QTL (*Q<sub>Lr.ubo-7B.2</sub>*), with the allele for leaf rust resistance contributed by Colosseo, was mapped in the distal region of the 7BL chr. 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. The QTL was detectable across the complete infection cycle, with  $R^2$  values for LRS ranging from 49.8% at the early stage of disease development (i.e. kernel milk stage, Zadoks 75) to 76.9% in the late phase (i.e. end of grain-filling, Zadoks 80). Using AUDPC, a very high QTL  $R^2$  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% for LRS at the early and 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<sup>-1</sup> in favour of the RILs carrying the resistance allele from Colosseo, respectively. Although a total of four QTLs were detected for HD, none of them was located in the chr. region harbouring *Q<sub>Lr.ubo-7B.2</sub>* (data not shown).

A total of 29 markers (1 EST-SSR, 5 SSRs and 23 DArTs) were mapped in the *Q<sub>Lr.ubo-7B.2</sub>* region within a 50 cM-wide interval; based on these markers, the QTL peak was clearly positioned in the distal region of the linkage group. Table 1 reports the results of the simple regression analysis using LRS (late disease stage) and AUDPC for those markers mapped in the QTL region. The LOD peak region, near to *Xgwm344.2*, included several DArT markers. A slight decrease in significance was observed at the end of the linkage group.

Three additional minor QTLs were identified by means of selective CIM analysis carried out on a subset of 76 lines with the molecular haplotype homogeneous to Lloyd at the *Q<sub>Lr.ubo-7B.2</sub>* significance (LOD 3.0) chr. interval. At two of the three QTLs, the resistance allele was contributed by Lloyd (*Q<sub>Lr.ubo-2A</sub>* in the distal region of chr. arm 2AL and *Q<sub>Lr.ubo-3A</sub>* in the proximal region of chr. 3AS), while at the third QTL (*Q<sub>Lr.ubo-7B.1</sub>* in the proximal region of chr. 7BS) the resistance allele was contributed by Colosseo. Each of the three QTLs showed an additive effect equal to ca. 5% for LRS and ca. 50 units for AUDPC.

The resistance of Colosseo was also expressed at the seedling stage. Colosseo showed an average IT value = 3 (resistance) with 14 out of the 16 Italian leaf rust isolates tested. Two isolates were clearly virulent to Colosseo/Creso (IT response = 8, similar to that showed by Lloyd). One isolate avirulent to Colosseo was used to characterize the complete set of RILs at the seedling stage. Heritability of the IT score was equal to 91.9%. Similarly to what was found in the open field experiment, the resistance at the seedling stage was controlled by a major QTL. This QTL, with a LOD peak value equal to 32.8, explained the 58.9% of the phenotypic variation; the QTL significance interval and LOD peak position were coincident with the *Q<sub>Lr.ubo-7B.2</sub>* ones.

### *Validation of Q<sub>Lr.ubo-7B.2</sub> on leaf rust resistance in a panel of lines related to Creso.*

The effects of *Q<sub>Lr.ubo-7B.2</sub>* were investigated in an association study based on a 2-year field data of a panel of 62 Creso-related lines. In the advanced lines, the LD among the set of loci mapping in the 7BL region showed highly significant  $P$  values and high  $D'$  values (> 0.3-0.5) over distances of 10 cM or more. Thus, the number of markers was adequate to validate the results obtained with the C × L RIL population.

The results of the association analysis over two years agreed with those obtained from the C × L population. Out of 14 SSRs spanning the 7BL distal chr. region, only those located within the *Q<sub>Lr.ubo-7B.2</sub>* significance interval were associated to leaf rust response. In particular, *Xwmc526.1*, *Xwmc526.2*, *Xbarc340.2*, *Xgwm146* and *Xgwm344.2* showed a significant association to both LRS and AUDPC, with the latest three markers showing the highest significance association level. At these markers, the alleles most probably inherited from Creso or its resistant derivatives were associated to the lower leaf rust infection level.

Our results indicate the presence of a major QTL for leaf rust resistance at the seedling and adult plant stages in the deletion bin 7BL10-0.78-1.00, which is one of the regions of the wheat chr. group 7 with the highest density of EST loci. The distal region near to the telomeric end of chr. group 7 of wheat is known to be rich in resistance genes, resistance gene analogs (RGAs) and defence-response genes (Dilbirli et al., 2004).

Up to now, three important leaf rust resistance genes have been mapped in the distal regions of chr. group 7: *Lr19*, introgressed from a short terminal 7EL segment of

*Lophopyrum ponticum* (Sharma and Knott, 1966), and the two closely linked genes *Lr14a* and *Lr14b* (distal portion of chr. 7BL; Dyck and Samborski, 1970). Moreover, the mapping location (Herrera-Foessel et al., 2007b) of *Lr14a*, one of the few designated resistance genes that originated from *Triticum turgidum*, is coincident with that of *QLr.ubo-7B.2*. The availability of

precise genetic stocks for the above mentioned genes/QTL in a homogeneous genetic background could facilitate testing for allelism and gene postulation studies.

Table 1. Results of the simple linear regression analysis for all the SSR and DArT markers mapped in the 7BL chr. region harbouring the major QTL for leaf rust resistance (*QLr.ubo-7B.2*). The Adult plant leaf rust response data (infected leaf area index, LRS, and area under the disease progress curve, AUDPC) of the 176 C × L RILs have been used. The *P*-values are based on the *F* test and the *b* coefficients are the slope coefficients of the linear regression equation.

Markers	Chr. position (cM)	LRS-late (%)			AUDPC (units)		
		<i>P</i>	LOD	<i>b</i>	<i>P</i>	LOD	<i>b</i>
<i>Xbarc340.2</i>	209.8	****	23.11	14.37	****	20.95	96.63
<i>Xgwm146</i>	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
<i>Xgwm344.2</i>	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

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