

A major QTL in the distal region of chromosome arm 7BL controls durable leaf rust resistance in durum wheat

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Introduction

Leaf rust is a threat to durum wheat (*Triticum turgidum* L. var. *durum*) production (Pasquini et al. 1997; Singh et al. 2004; Herrera-Foessel et al. 2006). We targeted the genetics of the resistance to *Puccinia triticina* conferred by the durum wheat Creso, an Italian cultivar released in 1974 that represents an important source of durable resistance to leaf rust in durum wheat under field conditions. This resistance has remained effective since 1975 in a wide range of environments (Pasquini and Casulli 1993; Martinez et al. 2007; Amaro et al., 2007).

Materials and Methods

Genetic materials:

- 176 recombinant inbred lines (RILs, F_{6,8}) from Colosseo (leaf rust resistant cv.; *Mexa's mutant* × Creso,) × Lloyd (leaf rust susceptible cv.; Cando × Emdore, NDSU);
- 62 F_{6,8} breeding lines with complex pedigrees related to Creso (donor of leaf rust resistance).

Phenotyping:

- The genetic materials were tested for both adult plant and seedling resistance.
- Adult plant resistance: open field experiments (3 reps each) in 2006 and 2007 with artificial inoculation (mixture of 16 *Puccinia triticina* Italian isolates). Measured traits: percentage of infected leaf area (leaf rust score, LRS, evaluated using the modified Cobb scale, Peterson et al. 1948, with three readings through the disease developmental cycle) and area under disease progress curve (AUDPC).
- Seedling resistance: greenhouse experiments (3 reps each) with single isolate. Measured trait infection type (IT) using the McNeal 0-9 scale.

Molecular analysis:

- RILs: 213 SSR and DArT markers grouped into 19 linkage groups. Total map length: 2022 cM (Mantovani et al. 2008).
- Panel of accessions for association analysis: a total of 57 SSRs, 43 of which evenly distributed for population structure evaluation and 14 mapping in the 7BL chr. region harbouring the major QTL as identified with the RILs). Composite interval mapping (CIM; Zeng 1994) was used to search for QTLs.
- EST genetic mapping:
 - rice genes from the region between *Xbarc340.2* and *Xgwm344.2* were used in BLASTn searches to identify wheat ESTs mapped on 7BL10-0.78-1.00.
 - PCR primers were designed with the software PRIMER3 available at <http://frodo.wi.mit.edu> and used on genomic DNA samples of *T. durum* cv. Langdon, *T. dicoccoides* accession Israel A and Chinese Spring nulli-tetrasomic (CS-NT) chr. substitution lines.
 - PCR amplicons were run on 4.5% polyacrylamide gels to identify polymorphisms.

Results

Adult plant, open field evaluation

Both LRS at three stages and AUDPC showed high heritability (from 72 to 83%).

One major QTL (*QLr.ubo-7B.2*) was found, using all the RILs, in the distal region of chr. 7BL (wheat deletion bin 7BL10-0.78-1.00), within a 5 cM interval (LOD - 2 supporting interval) flanked by *Xbarc340.2* and *Xgwm146* (upper part) and by *Xgwm344.2* (lower part). The resistance allele was from Colosseo.

QLr.ubo-7B.2 was detectable across the complete infection cycle, with LRS R² values ranging from 49.8% at the early stage of disease development (Zadoks 70) up to 76.9% in the late phase (Zadoks 80). AUDPC showed a QTL R² value = 72.9% and LOD peak equal to 44.5.

Additive effect ranged from 6.6 to 18.4% for LRS (early and late stage).

QLr.ubo-7B.2 did not show any concomitant effect on heading date.

Seedling response

Colosseo showed an average IT = 2 - 3 (resistance) with 14 out of 16 isolates.

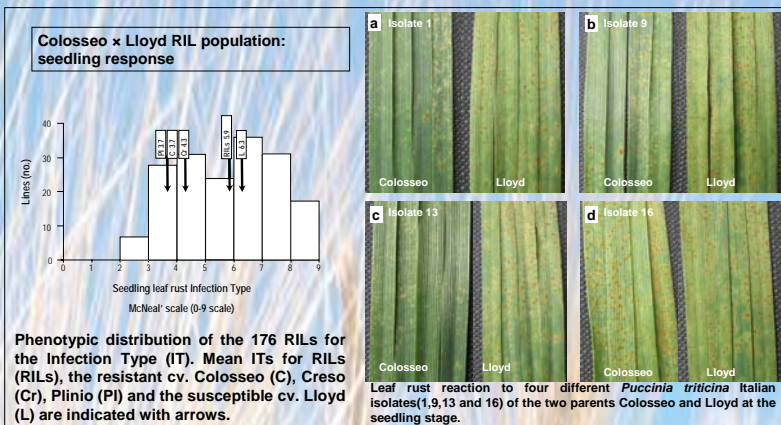
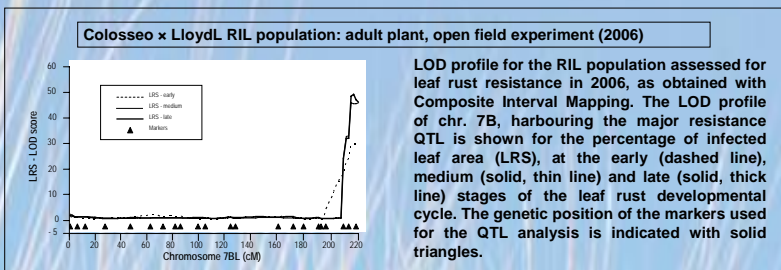
Two isolates were clearly virulent to Creso/Colosseo (IT = 8, similar to Lloyd).

One isolate avirulent to Colosseo was used to characterize for seedling response the complete set of RILs. IT score heritability was 91.9%. The genetic control of the resistance at the seedling stage was similar to what found in the open field experiment, with a major QTL (explaining 58.9% of the phenotypic variation and a LOD peak value = 32.8) coincident with *QLr.ubo-7B.2*.

Validation through association mapping in an independent multiple cross population

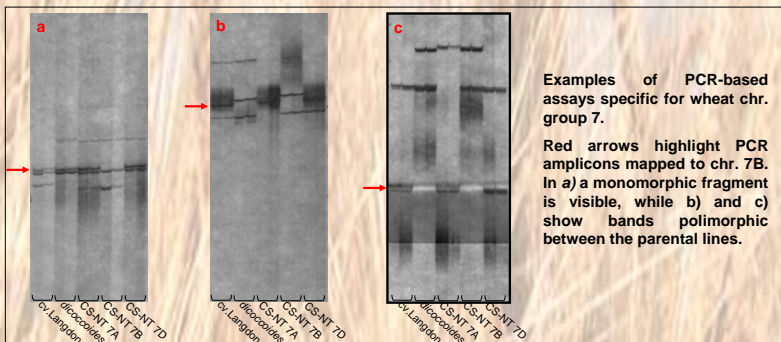
Out of 14 SSRs spanning the 7BL distal chr. region, only those located within the *QLr.ubo-7B.2* significance interval were associated to leaf rust response in both years of field experiments (2006 and 2007).

Markers *Xbarc340.2*, *Xgwm146* and *Xgwm344.2* showed the highest significance association level. At these markers, the alleles most probably inherited either directly from Creso or indirectly through Colosseo or Plinio were associated to a lower leaf rust infection. In particular, the marker with the highest association level to leaf rust resistance was *Xgwm146*, with LRS R² equal to 36.6 and 35.9% in 2006 and 2007, respectively.



Development of PCR markers

Colinearity has been reported between the distal portion of rice 6L and the distal ends of wheat group 7 chrs. The wheat ESTs on 7BL10-0.78-1.00 with a corresponding gene on rice 6L were selected to develop PCR markers for fine mapping of *QLr.ubo-7B.2*. Rice annotations were exploited to identify exon/intron boundaries, so PCR primers could be designed from exons to amplify predicted wheat genomic fragments spanning introns. Chinese Spring nulli-tetrasomic (CS-NT) chr. substitution lines were used to map amplicons to chr. 7B. Out Of 19 primer pairs, 7 detected polymorphism between Langdon and the *T. dicoccoides* accession Israel A. No polymorphism was present between the elite parents Colosseo and Lloyd.



Conclusions

Up to now, three important leaf rust resistance genes have been mapped in the distal regions of chr. group 7: *Lr19*, from *Lophopyrum ponticum* and the closely linked genes *Lr14a* and *Lr14b*. *Lr14a* is one of the few designated resistance genes originated from *Triticum turgidum* and its mapping location (Herrera-Foessel et al., 2007b) is coincident with that of *QLr.ubo-7B.2*. The availability of precise genetic stocks for *Lr14a* and *Lr14b* in a homogeneous genetic background could facilitate gene postulation studies / testing for allelism.

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