UNIVERSITÀ DEGLI STUDI
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Food and agricultural science, technology and biotechnology School of Doctorate

XXX Cycle

Identification of common resistance loci of wheat to three rust species by GWAS and multi-location trials

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Wheat is one of the most important cultivated crop worldwide.

Durum wheat (*Triticum turgidum ssp. durum*) is the only tetraploid species with high economical and agricultural importance, because of its use for human consumption.

*Italy: intense durum wheat breeding activity*
BIOTIC STRESSES

Fungal Infections

World population and food requirement

Food security
# RUST IN WHEAT

<table>
<thead>
<tr>
<th><strong>Puccinia graminis</strong></th>
<th><strong>Puccinia triticina</strong></th>
<th><strong>Puccinia striiformis</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>STEM RUST</strong></td>
<td><strong>LEAF RUST</strong></td>
<td><strong>STRIPE RUST</strong></td>
</tr>
<tr>
<td>(Black rust)</td>
<td>(Brown rust)</td>
<td>(Yellow rust)</td>
</tr>
</tbody>
</table>

- More than 60 *Sr* genes
- More than 72 *Lr* genes
- More than 70 *Yr* genes
Rust resistance in a tetraploid wheat collection

This study evaluated the resistance to rust disease in a tetraploid wheat collection (*Triticum turgidum* ssp *durum*) of 230 inbred lines. The whole collection consists of 128 durum wheat varieties plus 102 wild and domesticated accessions as reported in Laidò *et al.*, 2013; 2014.

**Genome wide Association mapping (GWAS)**

<table>
<thead>
<tr>
<th>Classification of the Tetraploid wheat collection (AABB)</th>
<th>Hulled/Naked</th>
<th>N° of accessions</th>
</tr>
</thead>
<tbody>
<tr>
<td>MacKey (1988)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. turgidum</em> ssp. <em>durum</em></td>
<td>Naked</td>
<td>128</td>
</tr>
<tr>
<td><em>T. turgidum</em> ssp. <em>turanicum</em></td>
<td>Naked</td>
<td>20</td>
</tr>
<tr>
<td><em>T. turgidum</em> ssp. <em>polonicum</em></td>
<td>Naked</td>
<td>20</td>
</tr>
<tr>
<td><em>T. turgidum</em> ssp. <em>turgidum</em></td>
<td>Naked</td>
<td>19</td>
</tr>
<tr>
<td><em>T. turgidum</em> ssp. <em>carthlicum</em></td>
<td>Naked</td>
<td>12</td>
</tr>
<tr>
<td><em>T. turgidum</em> ssp. <em>dicoccum</em></td>
<td>Hulled</td>
<td>19</td>
</tr>
<tr>
<td><em>T. turgidum</em> ssp. <em>dicoccoides</em></td>
<td>Hulled</td>
<td>12</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>230</strong></td>
</tr>
</tbody>
</table>

96 of the Italian Breeding

Domesticated accessions 102 genotypes

Wild accessions
The aim of the project was the determination of the genetic basis of biological mechanisms of plant response to *Puccinia* by means of an Association Mapping approach, to identify:

- QTL for resistance to Leaf rust, Stripe rust and Stem rust;
- Common QTL between rusts;
- The co-localization between MTAs and known resistance genes/QTL from literature.
PHENOTYPING
MULTILOCATION TRIALS

Tetraploid Wheat Collection was tested in different environments in the world and in different growth conditions.

FIELD ↔ GROWTH CHAMBER ↔ NATURAL INFECTION ↔ ARTIFICIAL INFECTION
FIELD EXPERIMENTS (adult plant experiments)

ARTIFICIAL INFECTION

Mix of STEM RUST races: QFCSC, QTHJC, MCCFC, RCRSC, RKQQC, TPMKC designated by the North American wheat stem rust differential set.

Severity scale (Peterson et al., 2011)
BIOSAFETY LEVEL 3 (seedling experiments) Plant pathology research facility
St. Paul campus

Facility containment for research on economically and ecologically important plant pathogens of agronomic and horticultural crops, nursery and forest industries and other resources of the State without risk of introducing the pathogens into Minnesota.
Materials and Methods

1. Planting
2. Inoculum preparation
3. Inoculation
4. Incubation
5. Disease scoring
SEEDLING EXPERIMENTS

STRIPED RUST

Pstv-14
Pstv-37
Pstv-40

STEM RUST

TTTTF
TKTTF
TRTTF
TPMKC
JRCQC

LEAF RUST

PSB14
Spain 5-2
Correlations graph of phenotypic data
90 K iSelect Infinium SNP array
Gatersleben, Germany  http://www.traitgenetics.com/en/

10% missing data, markers with minor allele frequency (MAF) of <0.1 were deleted.
High density consensus Map 0.6Mc/cM (Maccaferri et al. 2015)
Population structure

- The number of sub-groups (K) : 20 independent runs for each K (from 2 to 20)
- 100,000 Markov Chain Monte Carlo (MCMC) repetitions
- 100,000 burn-in period.
LINKAGE DISEQUILIBRIUM

Whole collection

Durum sub-sample

Q2

R programme (http://www.r-project.org)
GWAS

The whole collection, the durum subsample, and the Q2 group have been respectively mapped for which map information based on the durum wheat consensus map were used for the genome wide association mapping analysis. The ASSOCIATION MAPPING analysis was carried out with the mixed linear model (MLM) by Tassel software with three datasets:

- **a) MLM+Q+K**
- **b) MLM+K**

Significance of marker-trait associations (MTAs) was considered at threshold \(-\log_{10}(P\text{ value}) \geq 3.0\) based on a false discovery rate (FDR) of 0.05 or 0.1 (Mosig et al., 2001).
A total of 62 QTL were identified across datasets for the six races tested (JRCQC, TTTTF, TKTTF, TRTTF, TPMKC, TTKSK) in the seedling stage.

49 QTL single spot

- 28 JRCQC
- 3 TTTTF
- 2 TKTTF
- 11 TRTTF
- 2 TPMKC
- 3 TTKSK
QTLs FOR RESISTANCE TO THREE RUST SPECIES

A total of 30 common QTL between rusts were identified across datasets for ADULT PLANT

26 common QTL between 2 rusts
4 common QTL between 3 rusts

A total of 28 common QTL between rusts were identified across datasets for SEEDLING

27 common QTL between 2 rusts
1 common QTL between 3 rusts
Conclusions

• Several QTL regions for single rust across three different datasets (137 for leaf rust, 77 for stripe rust, 62 for stem rust) have been identified. Some of these regions were specific for dataset. In the last case, this could provide that some genetic subgroup could be particularly involved in the resistance.

• Study of the co-localization of QTL identified with genes known in the literature was carried out. Some of these genes observed were coincident with those identified in the previous studies, and some others were new.

• A total of 58 common QTL in both adult plant and seedling stage, have been identified between rusts. Mostly of these were in common between two rusts (53) and just a few between three rusts (5). Some of these QTL were specific for adult plant stage, some others for seedling stage.

• Interestingly, in some cases, the resistance was found to be associated with both growth stages. This is an interesting start point to understand some gene rusts complex which could be involved in the rusts resistance.

• This work could represent pivotal loci for breeding of new durably resistant varieties.
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