UNIVERSITÀ DEGLI STUDI
DI MODENA E REGGIO EMILIA

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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co-Supervisor: Giovanni Laidò

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.

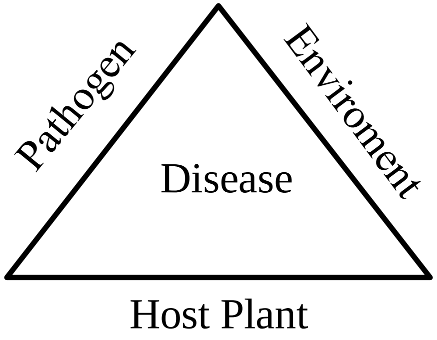
Italy: intense durum wheat breeding activity

World population and food requirement

Food security

BIOTIC STRESSES

FUNGAL INFECTIONS



Pathogen

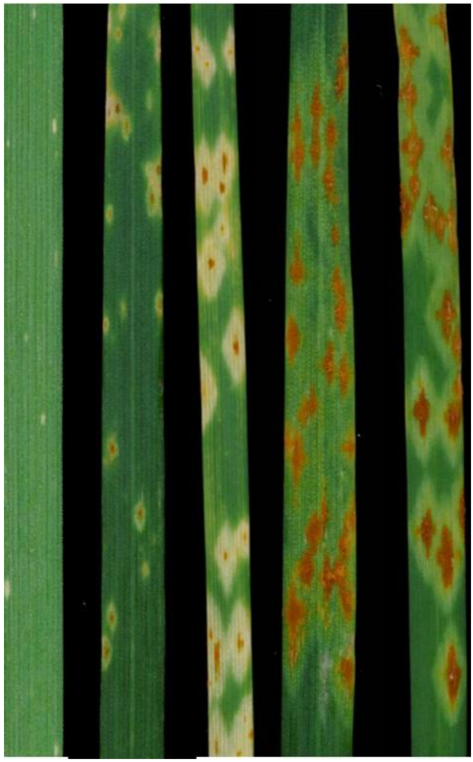
Enviroment

Disease

Host Plant

RUST IN WHEAT

Puccinia graminis
STEM RUST
(Black rust)



More than 60 *Sr* genes

Puccinia triticina
LEAF RUST
(Brown rust)

More than 72 *Lr* genes

Puccinia striiformis
STRIPE RUST
(Yellow rust)

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)

Classification of the Tetraploid wheat collection (AABB)	**Hulled/Naked**	**N°of accessions**
MacKey (1988)		
T. turgidum ssp. *durum*	Naked	128
T. turgidum ssp. *turanicum*	Naked	20
T. turgidum ssp. *polonicum*	Naked	20
T. turgidum ssp. *turgidum*	Naked	19
T. turgidum ssp. *carthlicum*	Naked	12
T. turgidum ssp. *dicoccum*	Hulled	19
T. turgidum ssp. *dicoccoides*	Hulled	12
Total		**230**

96 of the Italian Breeding

Domesticated accessions

Wild accessions

102 genotypes

AIM OF THE PROJECT

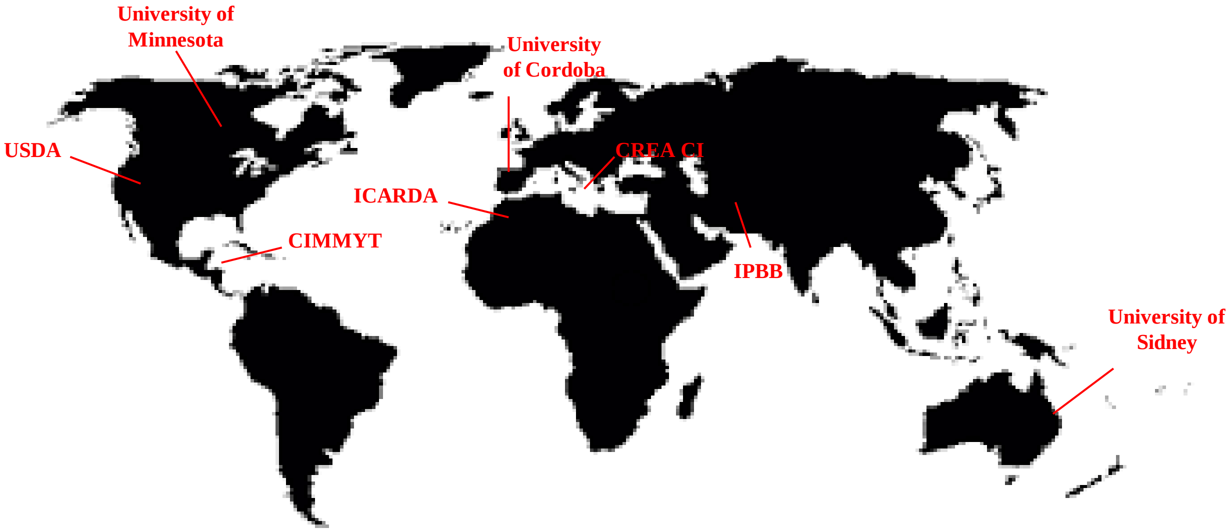
The aim of the project was the determination of the genetic basis of biological mechanisms of plant response to *Puccinia* by means of an Association Mapping approach, to identify:

✓ 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

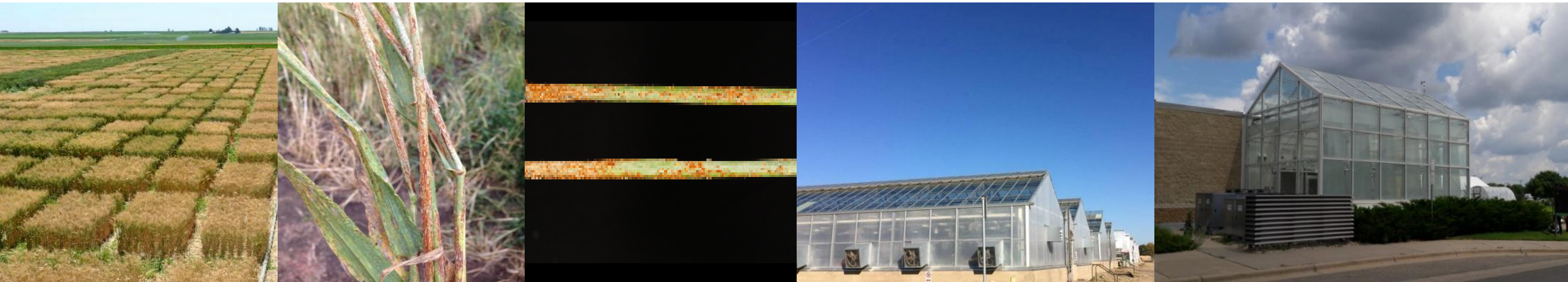
MULTILOCATION TRIALS

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



FIELD ⟷ **GROWTH CHAMBER**

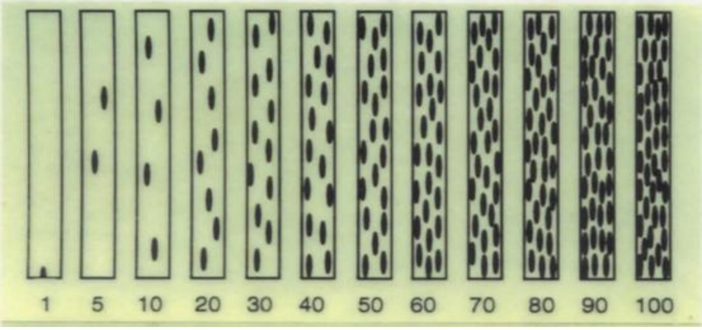
NATURAL INFECTION ⟷ **ARTIFICIAL INFECTION**



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)

UNIVERSITY OF MINNESOTA
Driven to Discover℠

USDA-ARS Cereal Desease Lab

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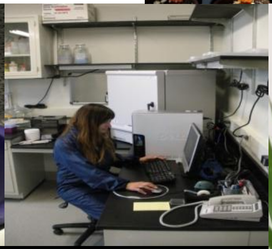
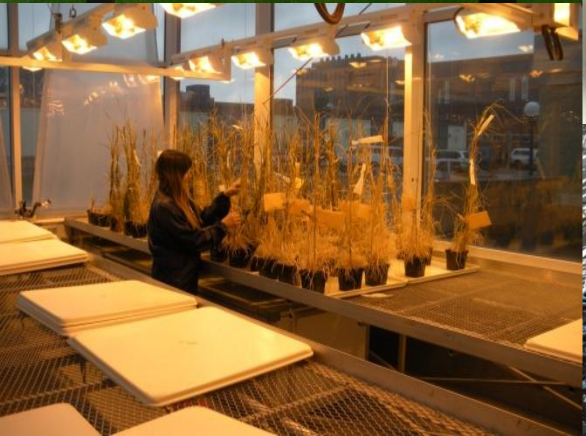
BIOSAFETY LEVEL 3 (seedling experiments)
Plant pathology research facility

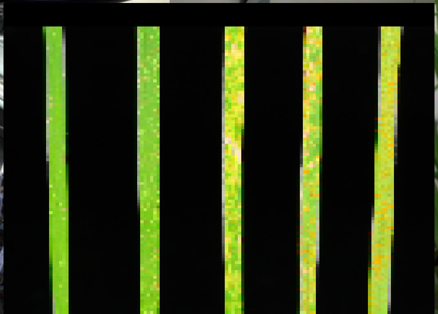
St. Paul campus

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

MINNESOTA DEPARTMENT OF AGRICULTURE



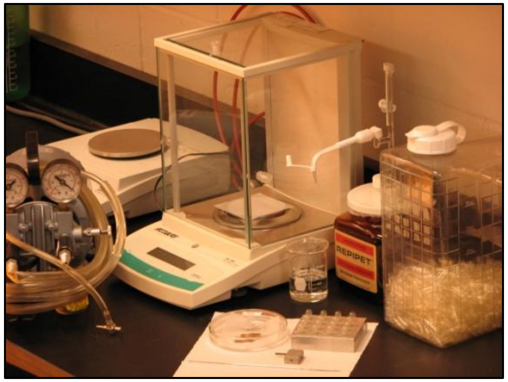




Materials and Methods

1. Planting

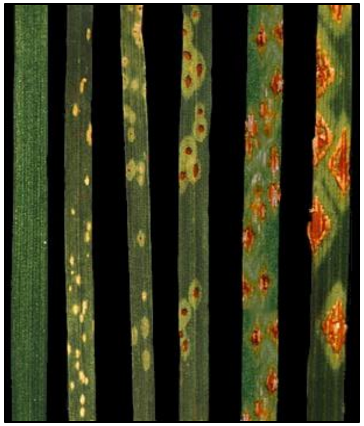
2. Inoculum preparation



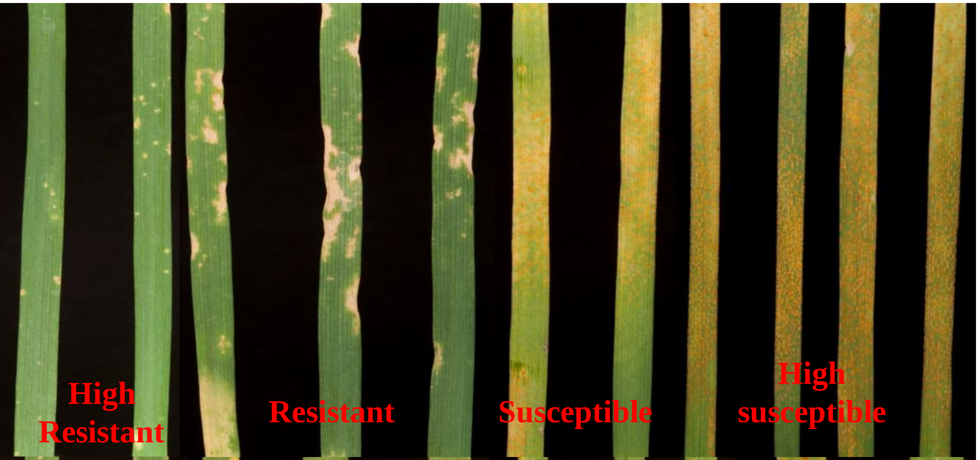
3. Inoculation

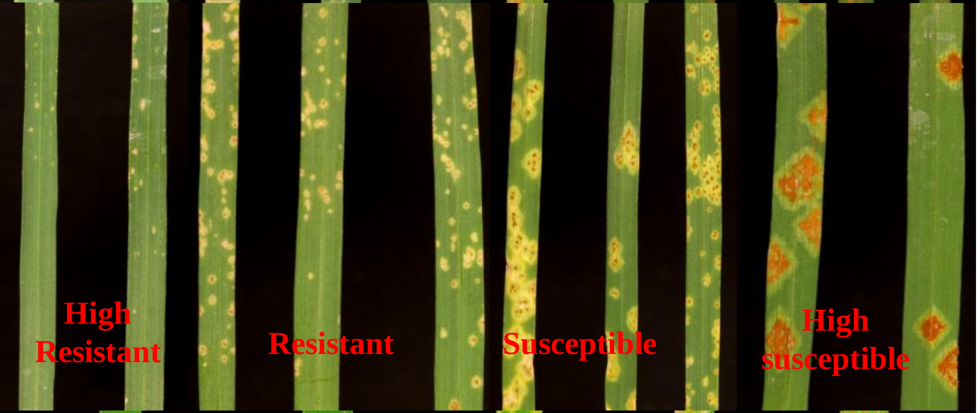
4. Incubation

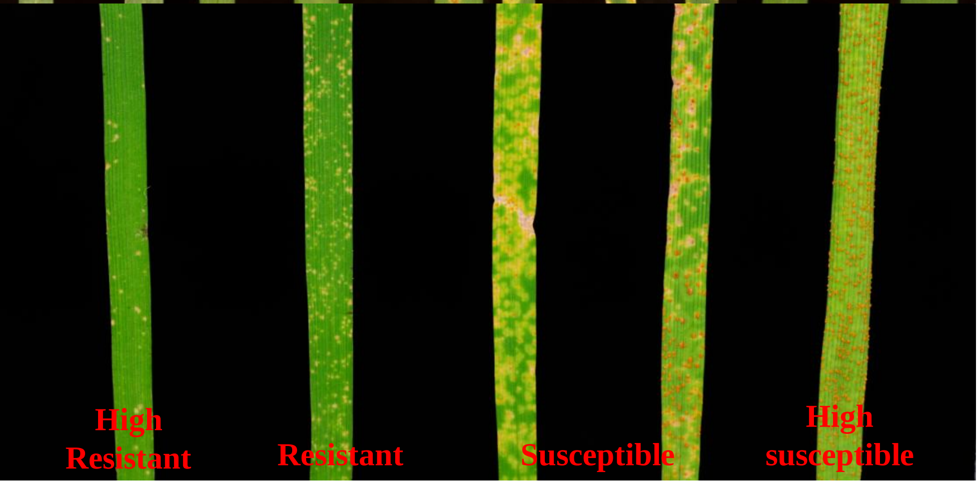
5. Disease scoring



SEEDLING EXPERIMENTS

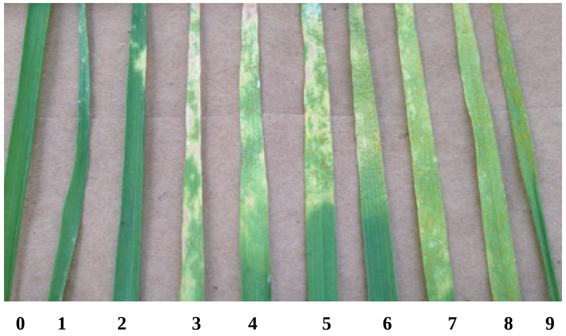






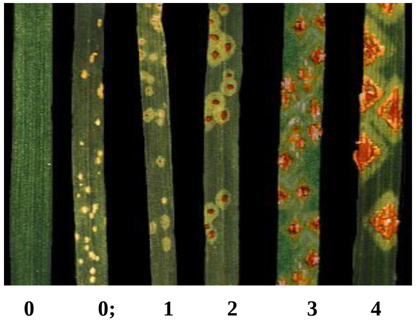
STRIPE RUST

Pstv-14
Pstv-37
Pstv-40



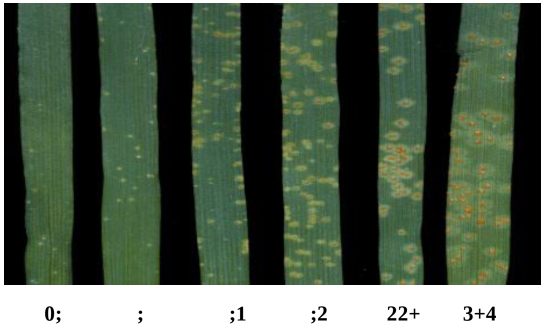
STEM RUST

TTTTF
TKTTF
TRTTF
TPMKC
JRCQC

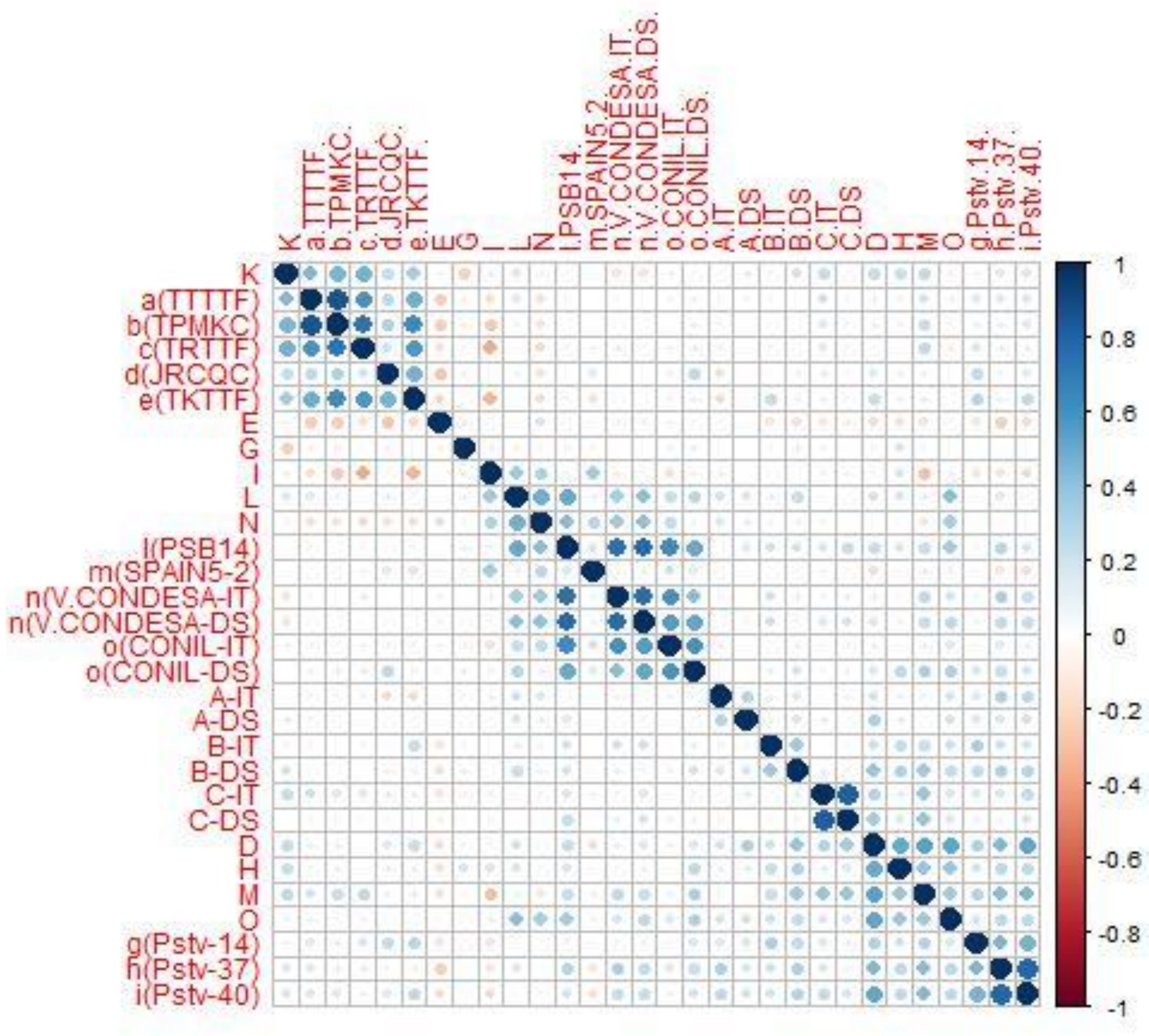


LEAF RUST

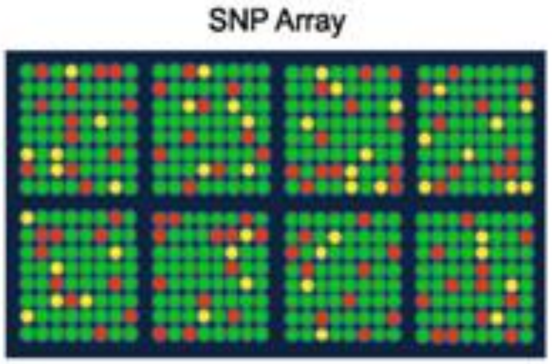
PSB14
Spain 5-2



Correlations graph of phenotypic data



GENOTYPING



- ✓ 90 K iSelect Infinium SNP array

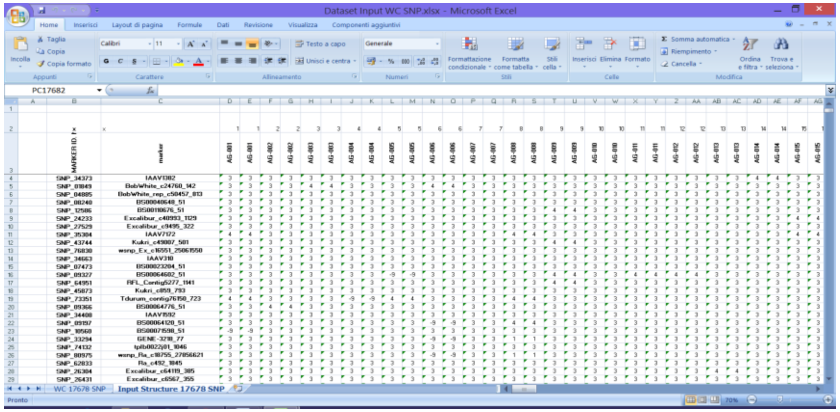
  Gatersleben,
  Germany http://www.traitgenetics.com/en/

illumina®

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.



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG
1																																	
2			x	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11	12	12	13	13	14	14	15	1
3		MARKER ID. x	marker	AG-001	AG-001	AG-002	AG-002	AG-003	AG-003	AG-004	AG-004	AG-005	AG-005	AG-006	AG-006	AG-007	AG-007	AG-008	AG-008	AG-009	AG-009	AG-010	AG-010	AG-011	AG-011	AG-012	AG-012	AG-013	AG-013	AG-014	AG-014	AG-015	AG-015
4		SNP_34373	IAAV1382	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	4	4	3	3
5		SNP_01849	BobWhite_c24760_142	3	3	3	3	4	4	3	3	3	3	4	4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
6		SNP_04885	BobWhite_rep_c58457_813	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
7		SNP_08240	BS00040648_51	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
8		SNP_12586	BS00110676_51	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	4	4	3	3	3	3	3	3	3	3	3	3	3	3
9		SNP_24233	Excalibur_c40993_1129	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	4	4
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24		SNP_33294	GENE-3218_77	3	3	3	3	3	3	3	3	3	3	-9	-9	3	3	1	1	3	3	3	3	3	3	3	3	3	3	3	3	3	3
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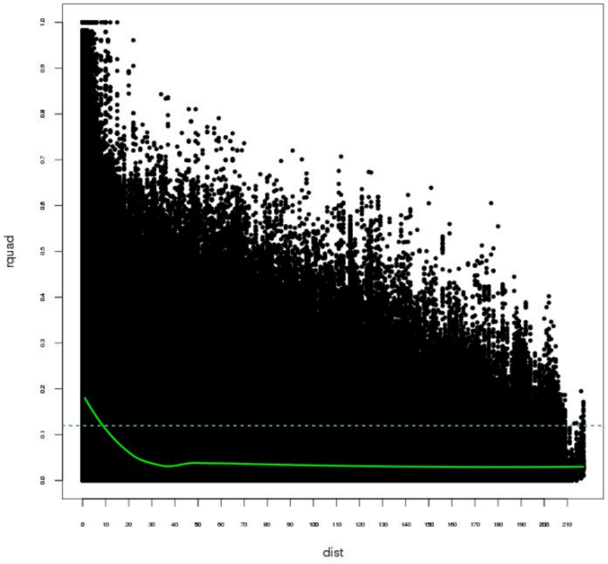
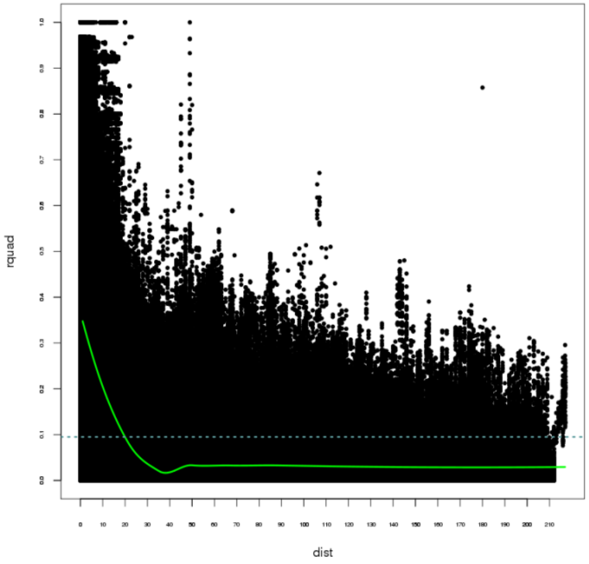
Structure software package 2.3

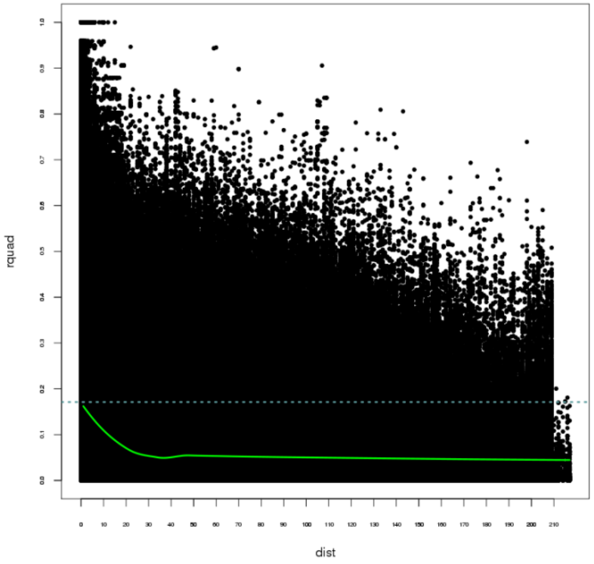
LINKAGE DISEQUILIBRIUM



Whole collection



Durum sub-sample



Q2

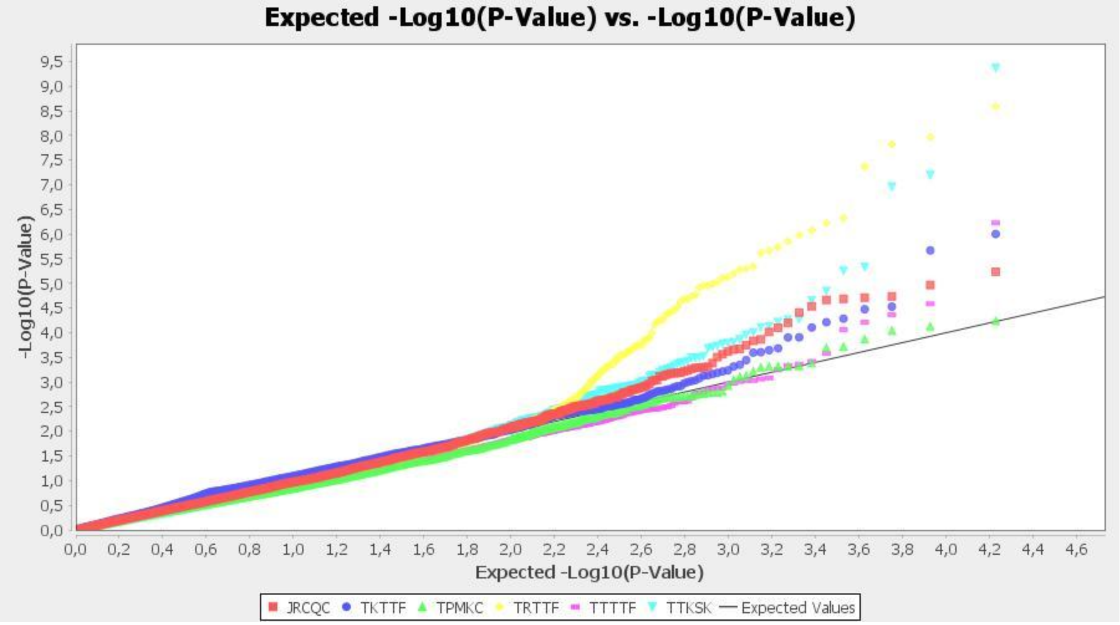
R programme (http:// www.r-project.org)

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



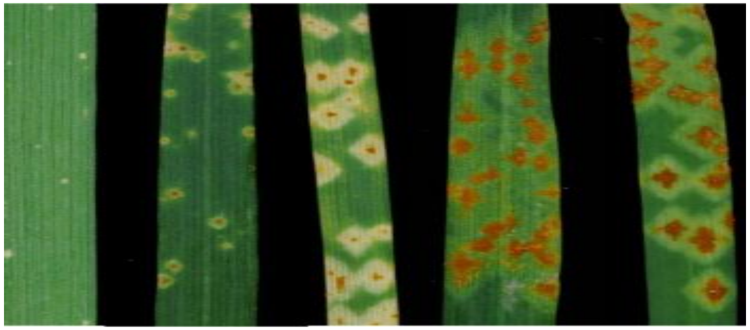
Significance of marker-trait associations (MTAs) was considered at threshold $-\log_{10}$ (P value) $\geq$ 3.0 based on a false discovery rate (FDR) of 0.05 or 0.1 **(Mosig et al., 2001).**

Tassel software 5.2. 39

STEM RUST RESISTANCE QTLs (seedling)

(Manuscript in preparation)

A total of 62 QTL were identified across datasets for the six races tested (JRCQC, TTTTF, TKTTF, TRTTF, TPMKC, TTKSK) in the seedling stage.



0 1 2 3 4 Infection Types (ITs)

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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Turupeskov Y. (IPBB, Kazakhstan)

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