Fine Mapping of the Chromosome 5B Region Carrying Closely Linked Rust Resistance Genes *Yr47* and *Lr52* in Wheat

Naeela Qureshi

Plant Breeding
Institute
University of Sydney
Australia

THE UNIVERSITY OF SYDNEY

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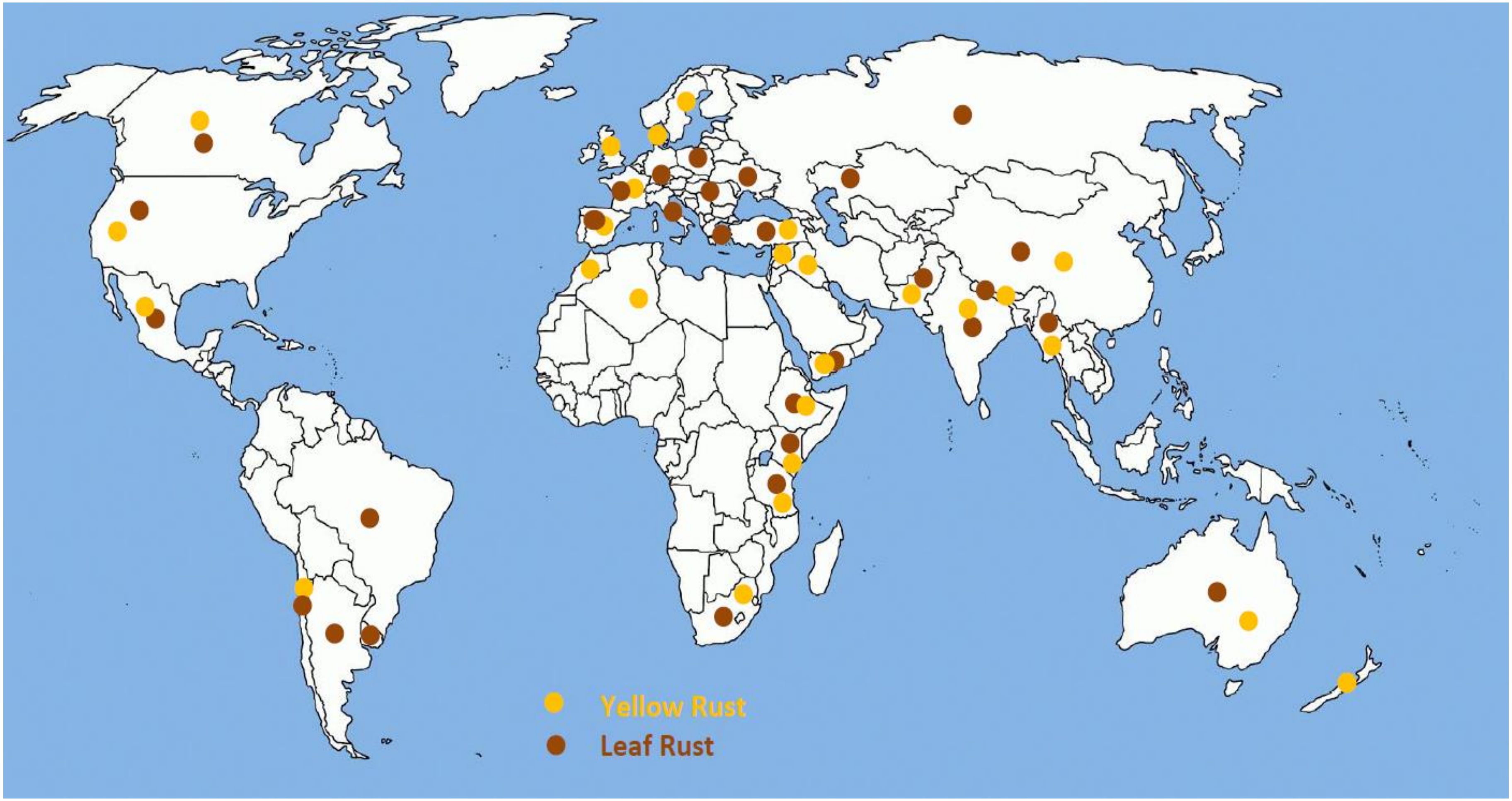
Wheat Rusts Damage

RUST: DID YOU KNOW ?

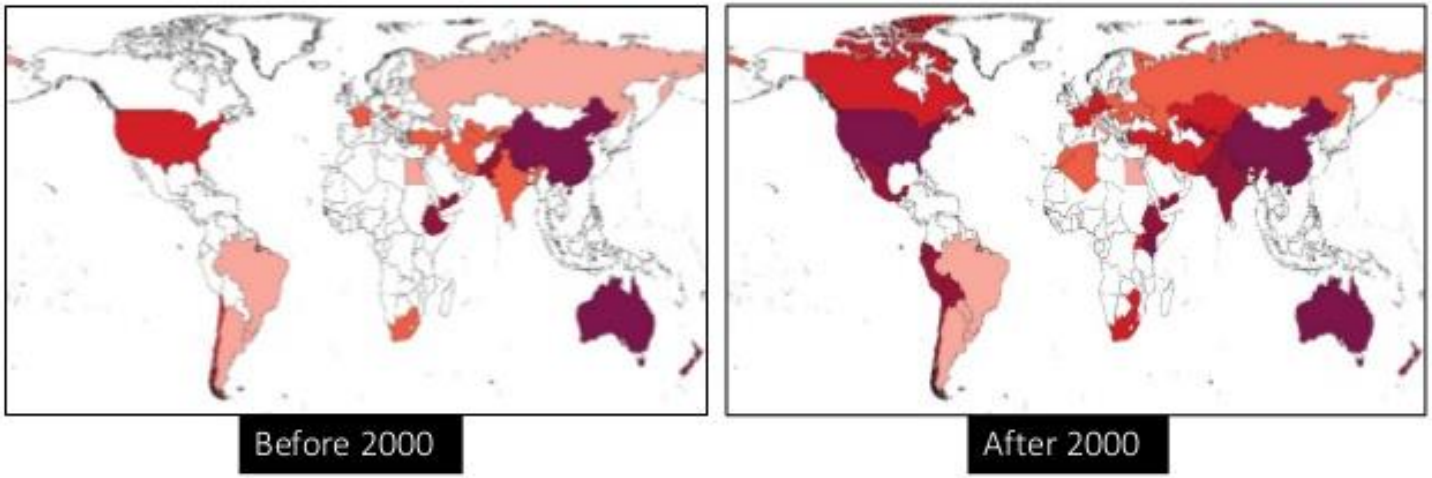
JUST ONE HECTARE OF RUST-INFECTED WHEAT CAN PRODUCE 19.1 KG OF RUST SPORES

WWW.RUSTBUST.COM.AU

Wheat Rust Prevalence



Historical and Current Stripe Rust threats



Before 2000

After 2000

0	Not recorded, or no response
1	Rare
2	Localized in some seasons
3	Localized in most seasons
4	Widespread in some seasons
5	Widespread in most seasons

- Historically, stripe rust has mainly been endemic only in cool climate regions
- In recent decades, stripe rust has spread rapidly to areas previously unaffected

Slide taken from Philip et al. BGRI 2014

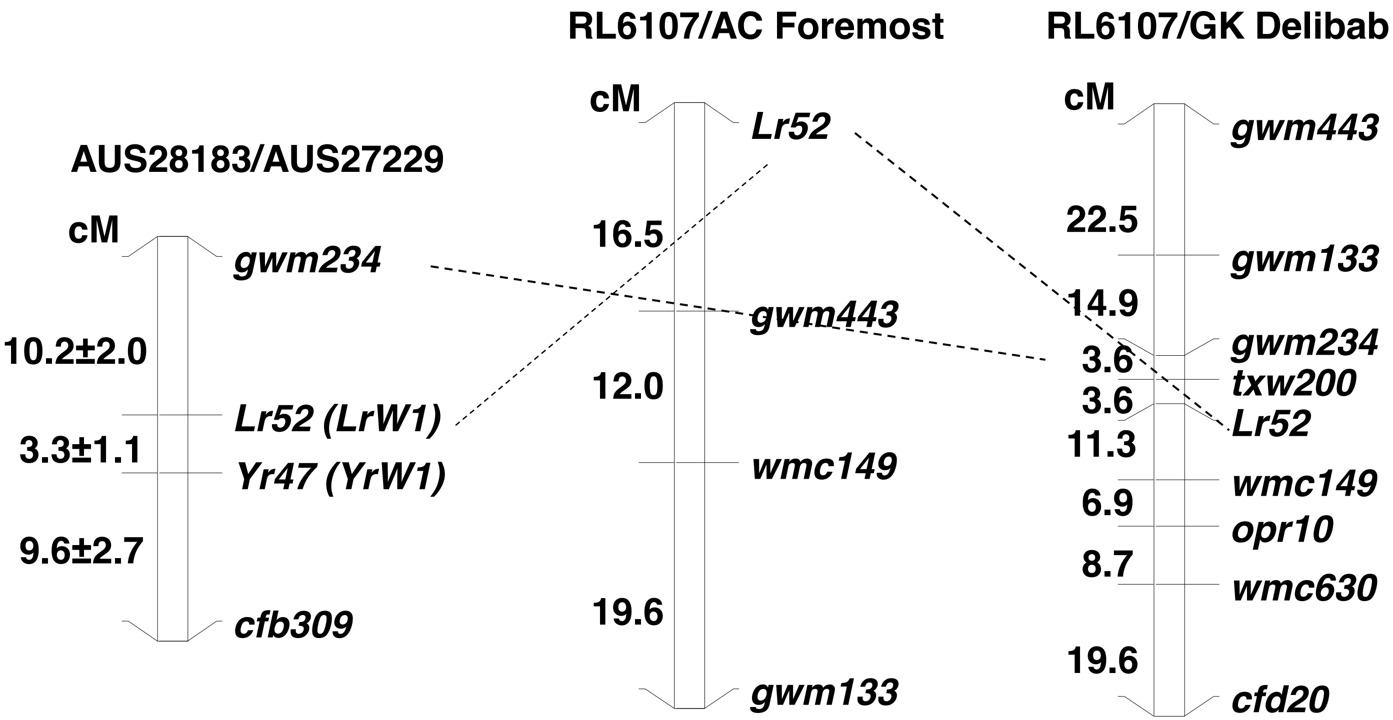
Wheat Rusts – Australian scenario

- ☐ Rust diseases have estimated to cause annual losses of A$139 million (Murray and Brennan 2009)

- Breeding for resistance is the best control strategy

Background

- Hiebert et al. (2005) :*Lr52*
- Bansal et al. (2011) :*Yr47*



Aim of the Study

☐ Linked resistance genes *Yr47* and *Lr52* are known to be effective against Australian, Indian, Kenyan and Canadian pathotypes of stripe rust and leaf rust pathogens

☐ To develop closely linked markers to *Yr47* and *Lr52* for marker assisted selection

Development of RIL

Recombinant inbred line (RIL) was developed (AUS28183/AUS27229)

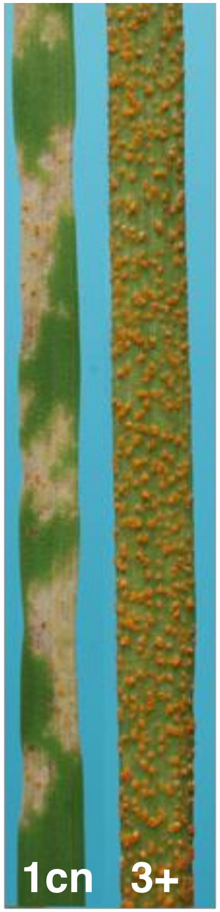
Phenotyping of RIL population

Pathotypes	Rust response	
	AUS28183	AUS27229
Stripe rust (134 E16A+ Yr17+ Yr27+)	1CN	3+
Leaf rust (104 1,2,3, (6), (7), 11,13)	0;-;1	3+

1= necrotic and chlorotic area with restricted sporulation
0= no visible uredinia
;= hypersensitive fleck
3+= abundance of sporulation without chlorosis

Leaf Rust

Stripe Rust



Genomic Resources used

- ☐ Chinese Spring Survey sequences
- ☐ EST based markers
- ☐ NBS-LRR gene sequences
- ☐ Physical map of chromosome 5BS
- ☐ iSelect 90K SNP Infinium Array
- ☐ SSR and STS markers available in public domain and derived from CSS contigs

Marker development

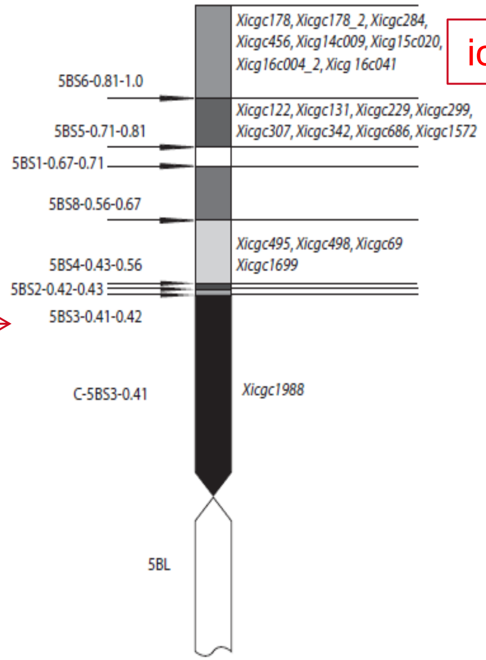
List of Brachy genes/CSS contigs physically anchored in chromosome arm 5BS

Orthologous Brachy genes	CSS Contigs
Bradi4g00200	Ta5BS-2254420
Bradi4g00230	Ta5BS-2268897
Bradi4g00250	Ta5BS-2255840
Bradi4g00310	Ta5BS-2265631
Bradi4g00320	Ta5BS-2242458
Bradi4g00330	Ta5BS-2231195
Bradi4g00370	Ta5BS-2240502
Bradi4g00380	Ta5BS-2252472
Bradi4g00410	Ta5BS-2249452
Bradi4g00430	Ta5BS-2254119
Bradi4g00450	Ta5BS-1452126
Bradi4g00460	Ta5BS-2252577
Bradi4g00490	Ta5BS-2294725
Bradi4g00530	Ta5BS-2288254
Bradi4g00550	**Ta5BS-2262954**
Bradi4g00620	**Ta5BS-2247303**
Bradi4g00670	Ta5BS-2246065
Bradi4g00720	Ta5BS-2255211
Bradi4g00730	Ta5BS-2252410
Bradi4g00740	Ta5BS-2259232
Bradi4g00750	Ta5BS-2242968
Bradi4g00760	Ta5BS-2276504
Bradi4g00790	Ta5BS-643948
Bradi4g00810	Ta5BS-2295731
Bradi4g00850	Ta5BS-2294109
Bradi4g00860	Ta5BS-2272737
Bradi4g00880	Ta5BS-2295555
Bradi4g00900	Ta5BS-2283682
Bradi4g00910	Ta5BS-2262359

Survey sequences representing orthologous region in *Brachypodium distachyon*

Marker development

Physical map chromosome arm 5BS



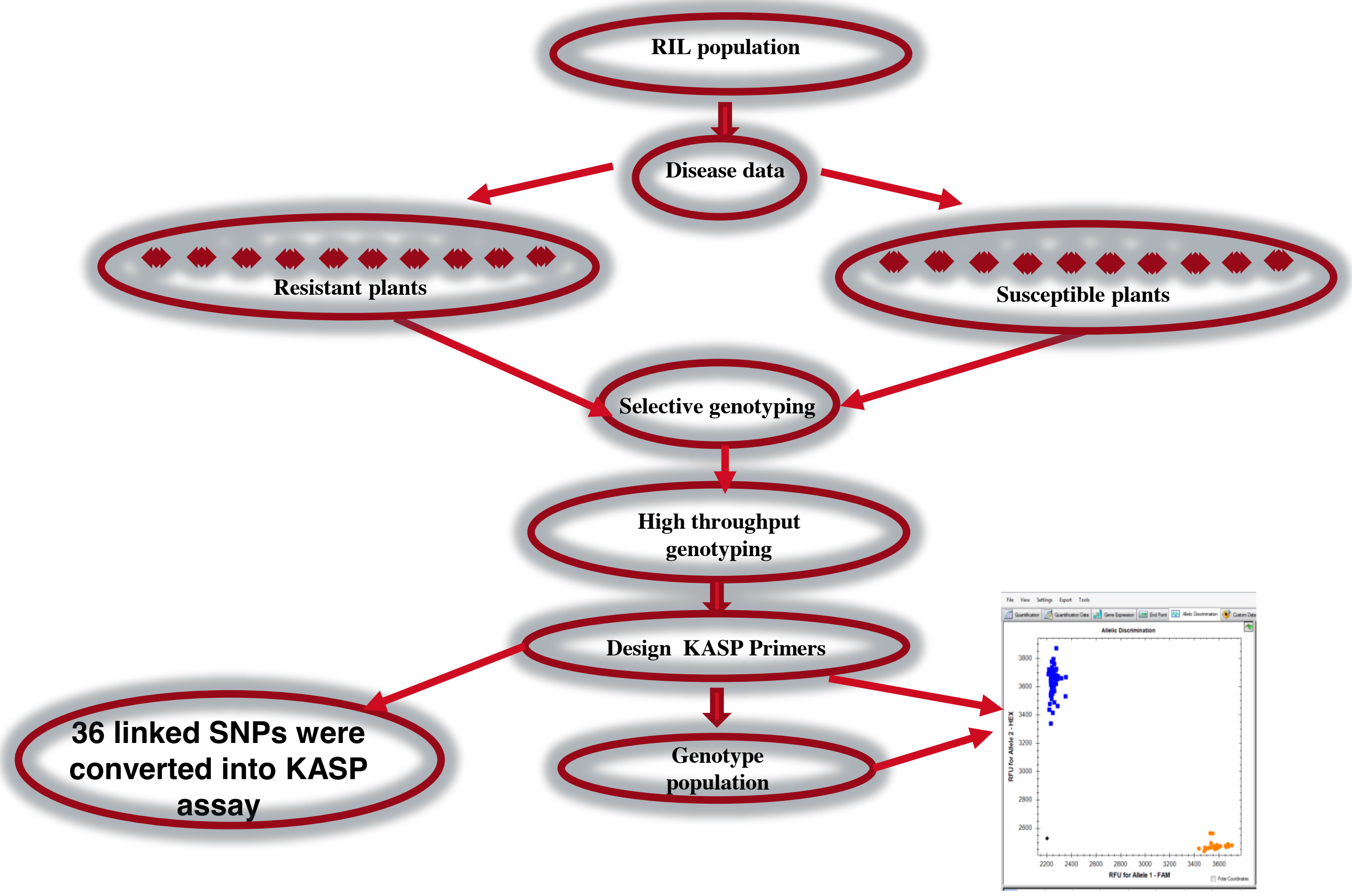
icg16c008

Fig. 2. Location of SSR markers in the deletion bins of chromosome 5BS.

- ☐ **81 SSR markers were developed**
- ☐ **25 markers were polymorphic**

Nesterov et al. 2015

High throughput genotyping



RIL population

Disease data

Resistant plants

Susceptible plants

Selective genotyping

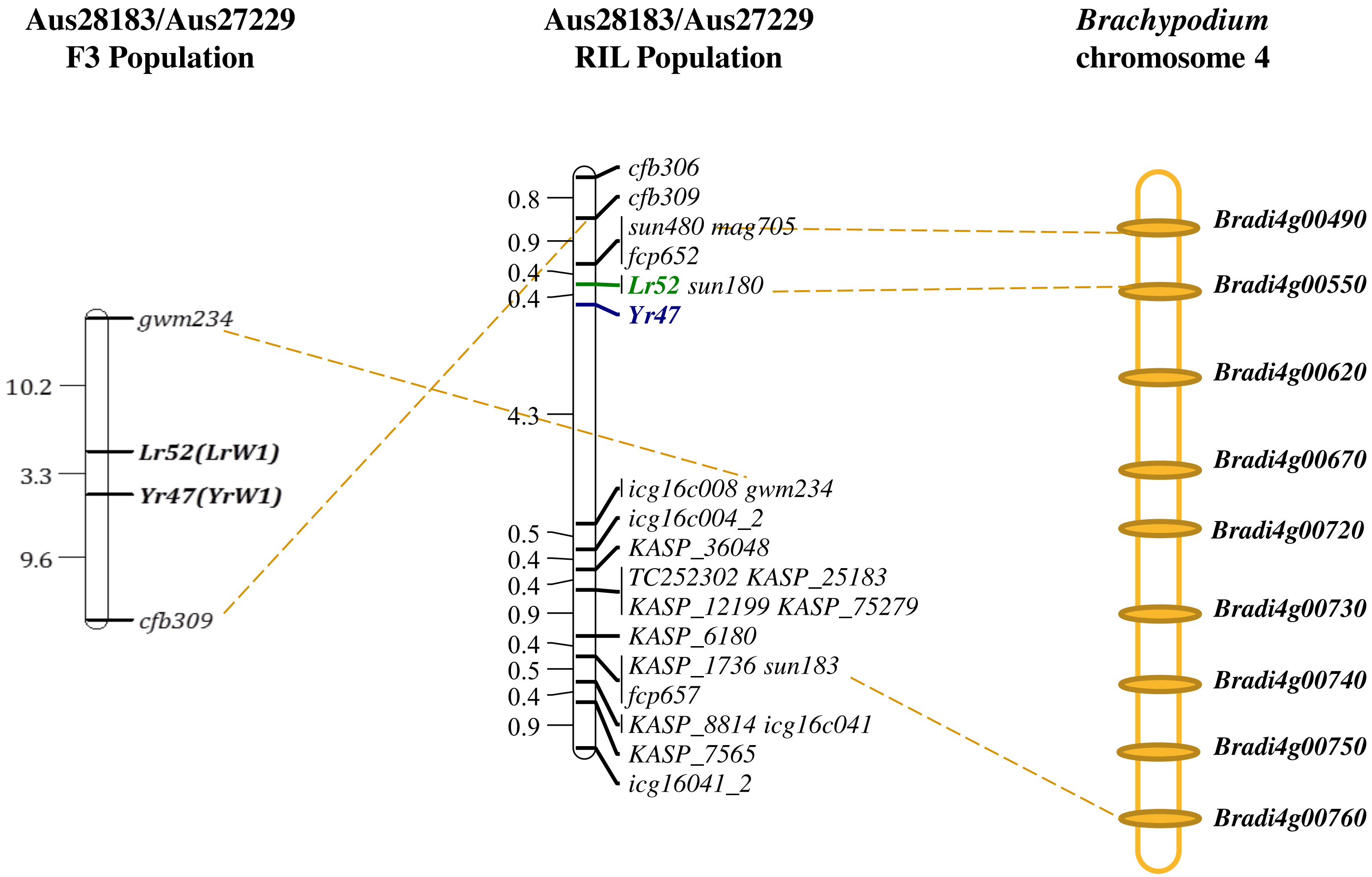
High throughput genotyping

Design KASP Primers

36 linked SNPs were converted into KASP assay

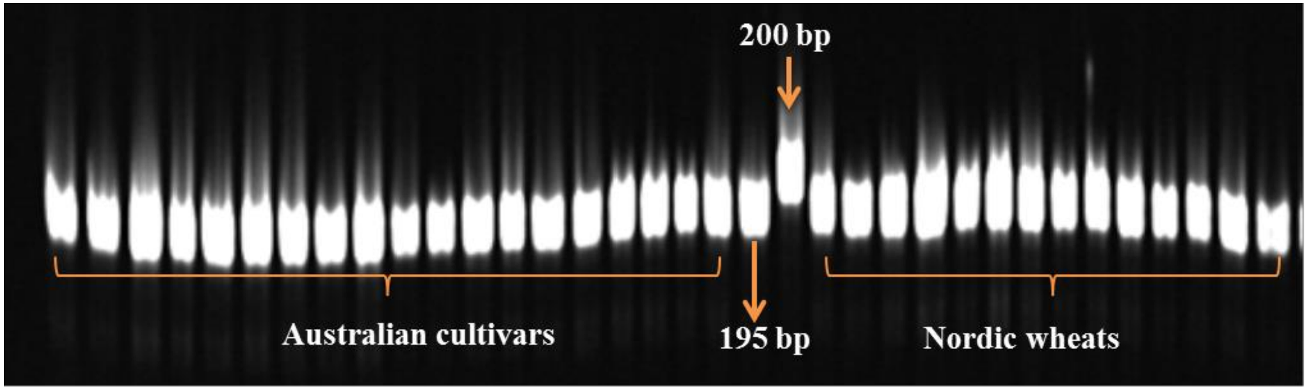
Genotype population

Genetic linkage map

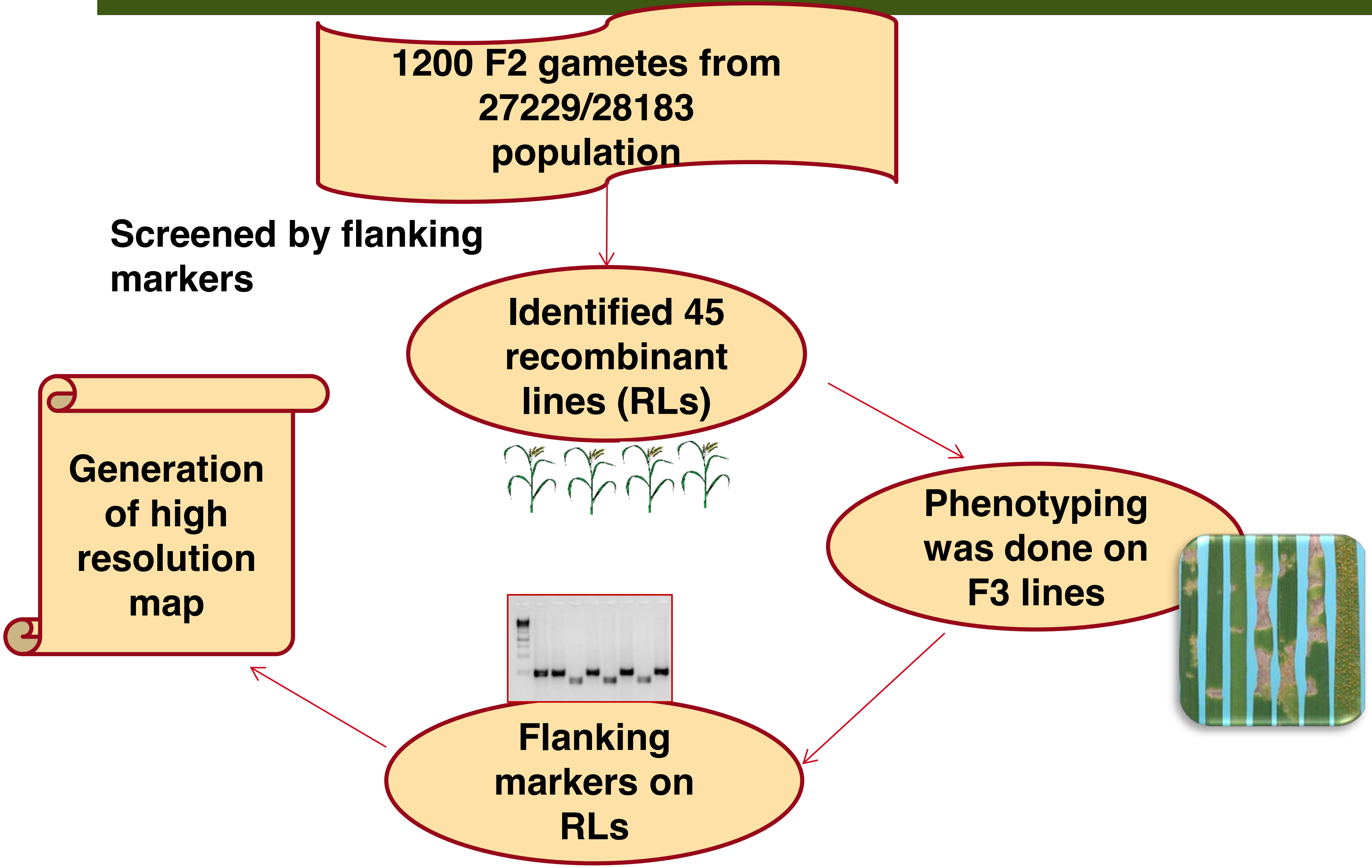


Marker Validation

- ☐ **Closely linked markers were tested on a set of**
  - ☐ 76 Australian cultivars
  - ☐ 121 Nordic wheat cultivars
  - ☐ Backcross derivatives
- ☐ **None of the varieties amplified *Yr47/Lr52* linked product**



High resolution linkage map



1200 F2 gametes from 27229/28183 population

Screened by flanking markers

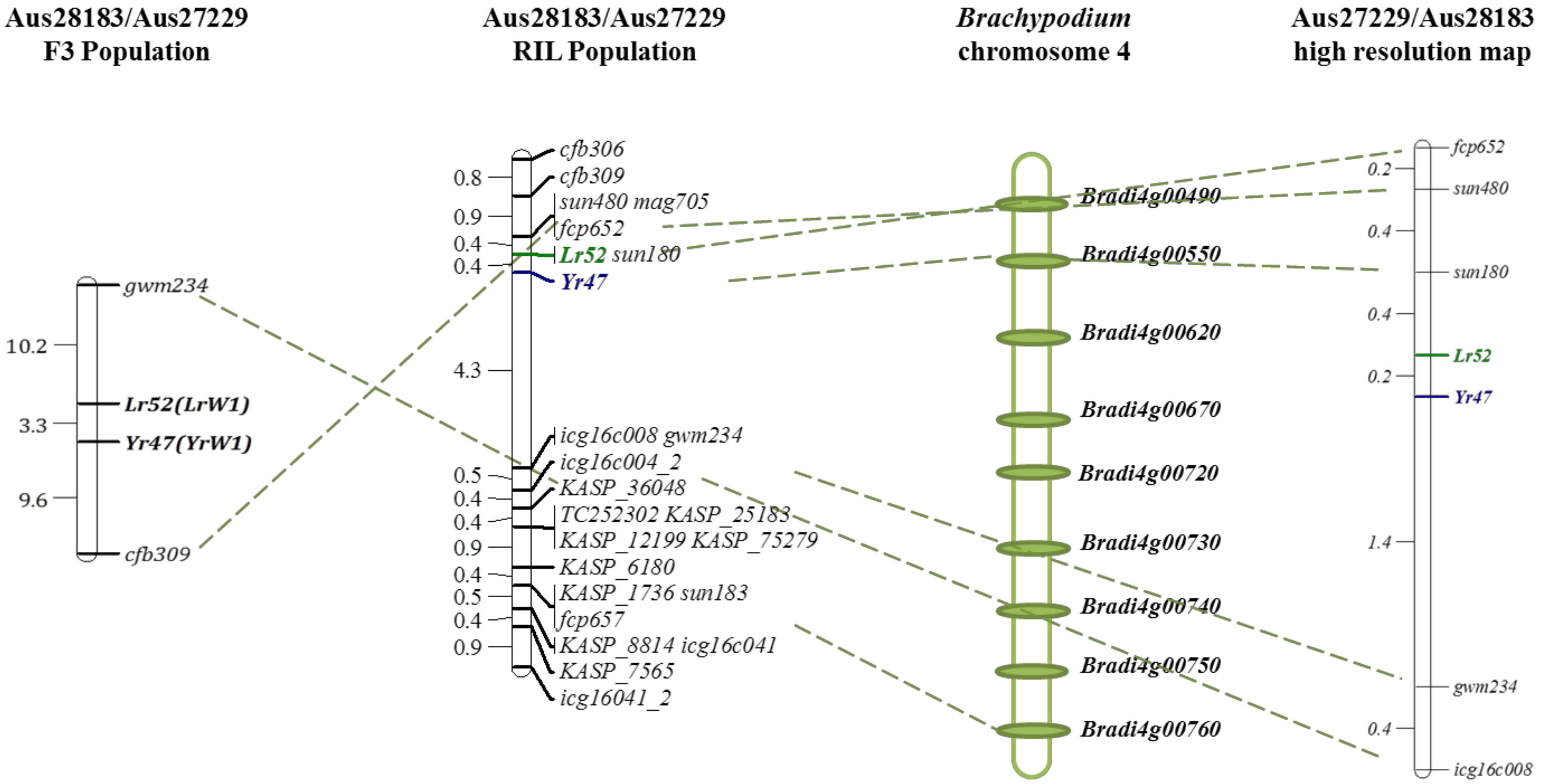
Identified 45 recombinant lines (RLs)

Phenotyping was done on F3 lines

Flanking markers on RLs

Generation of high resolution map

Genetic linkage map



Mutants for *Yr47/Lr52*

- ☐ **Mutants of these genes have been generated**
- ☐ **Three categories of mutants**

Mutants for Both	**Mutants for *Yr47***	**Mutants for *Lr52***
YRLR=S	**YR=S, LR=R**	**LR=S, YR=R**

TGACv1 assembly

TGAC assembly	Scaffold length	Brachy gene	CSS contig	CSS contig length
TGACv1.3_scaffold_424411_5BS	44,081	Bradi4g00490	Ta5BS-2294725	23115
TGACv1.3_scaffold_424851_5BS	24,539	Bradi4g00530	Ta5BS-2288254	
TGACv1.3_scaffold_424744_5BS	28,634	Bradi4g00550	Ta5BS-2262954	16749
TGACv1.3_scaffold_423631_5BS	112,171	Bradi4g00620	Ta5BS-2247303	15928
TGACv1.3_scaffold_423788_5BS	92,275	Bradi4g00670	Ta5BS-2246065	7485
TGACv1.3_scaffold_423189_5BS	409,517	Bradi4g00720	Ta5BS-2255211	10590
TGACv1.3_scaffold_423669_5BS	108,369	Bradi4g00730	Ta5BS-2252410	9036
TGACv1.3_scaffold_423296_5BS	211,800	Bradi4g00740	Ta5BS-2259232	12317
TGACv1.3_scaffold_423592_5BS	117,806	Bradi4g00750	Ta5BS-2242968	11116
TGACv1.3_scaffold_423463_5BS	148,473	Bradi4g00760	Ta5BS-2276504	8616

(TGACv1, http://plants.ensembl.org/)

NTGv0.3 genome assembly

- Most of the markers physically map to two which are adjacent to one another in NRGv1.0 Super Scaffold

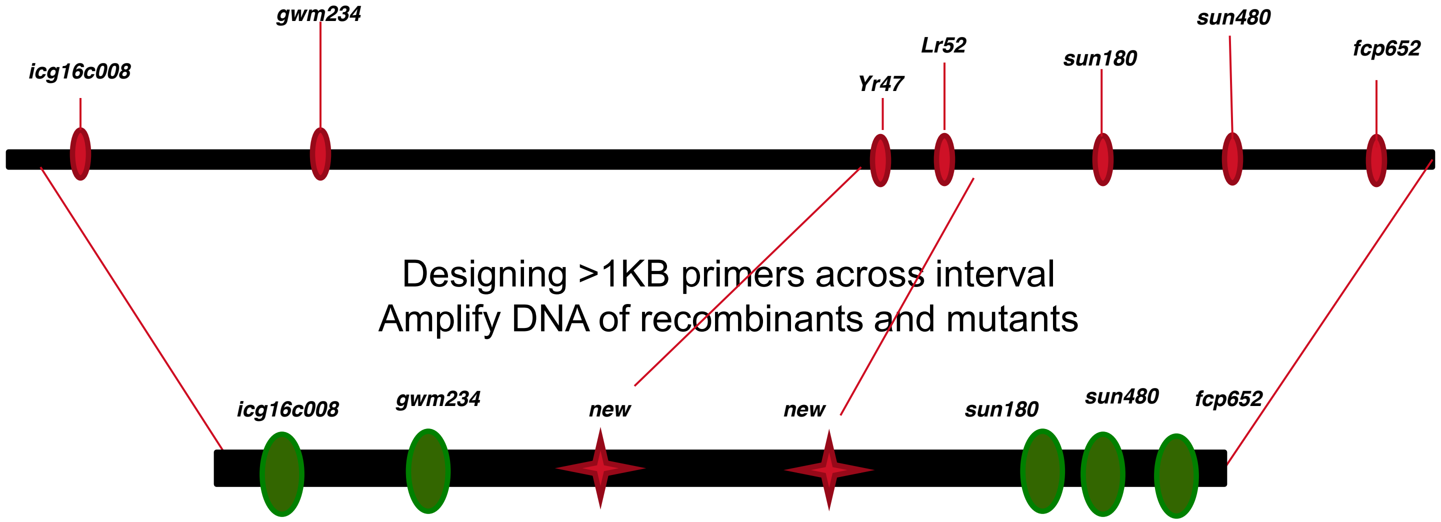
- Flanking markers fcp652, sun180 and icg6c008, physically map to one scaffold delimiting an interval of ~1.2Mbp

-

- There are up to 5 putative disease resistance genes located within this interval out of which two are in the region of interest

Future Work

Chromosome arm 5BS



Designing >1KB primers across interval
Amplify DNA of recombinants and mutants

NRGv1.0 Super Scaffold

Donor Development

Donor parent

Yr47/Lr52 **RIL**

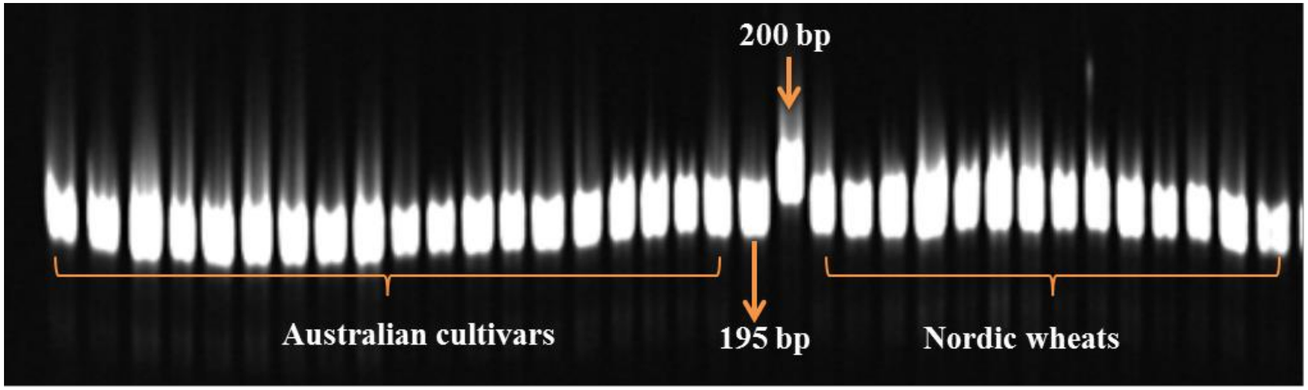
Recurrent parent

- **Ventura**
- **Emu Rock**
- **Mace**
- **Suntop**
- **Magenta**
- **Wyalkatchem**
- **QAL2000**
- **Calingiri**
- **Carinya**
- **Hume**
- **Yitpi**
- **Sunvex**

All resistant backcross derivatives carried the *Yr47/Lr52*-linked allele

Marker Validation

- **Closely linked markers were tested on a set of Yr47/Lr52 lacking cultivars:**
  - 76 Australian
  - 121 Nordic
  - Donors
- **None of these genotypes amplified *Yr47/Lr52*-specific product**



Conclusion

- **Availability of extensive genomic resources provided critical information for saturating *Yr47* and *Lr52* region**

- **Markers developed from CSS contigs (IWGSC) showed more polymorphism among all genomic resources and led to the development of closely linked marker *sun180***

- ***Yr47* and *Lr52* gene region is present in TGAC and NR gene assemblies which will help in cloning of these genes**

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