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Final report

project

Molecular markers for broadening the genetic base of stem rust resistance genes effective against strain Ug99

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1 Acknowledgments

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2 Executive summary

CSIRO component

The study focused on dissecting stem rust resistance in Thatcher (Tc) wheat and identifying genes that confer effective resistance against a broad spectrum of field races. Thatcher, an old American wheat, is a well-known source for durable resistance and has been studied extensively over several decades by North American researchers. Many of these classical genetic studies concluded that resistance was complex and difficult to transfer and utilise by breeders. More recently, groups at USDA conducted mapping studies of stem rust resistance in Tc and identified several QTLs. In this project we collaborated with colleagues at USDA and AgCanada to develop germplasm together that was then evaluated for stem rust resistance in multiple locations. This work identified a strong, broad spectrum stem rust resistance gene on chromosome 3B of Tc which co-located with one of the QTL previously identified by USDA colleagues. A tightly linked SNP-based marker was developed that can now be used by breeders worldwide. After many years of classical and molecular genetic analysis of Tc resistance, a marker is now available to select for a major component of durable stem resistance. This marker together with donor germplasm will be made available to Indian and Australian researchers as part of the ongoing marker Implementation project (CIM2013-009).

Project outputs depended on the global collaboration to combat the stem rust threat. Bringing together expertise in stem rust field evaluation and access to reliable field nurseries in three countries enabled the rapid identification of valuable resistance genes. We anticipate that stem rust resistance characterised in this project will be incorporated into adapted germplasm in India and Australia but also utilised by other companies that are breeding for stable stem rust resistance. The project also provided the opportunity for future collaborations with USDA and AgCanada aimed at identifying other components of stem rust resistance in Tc and tagging additional resistance genes with tightly linked, robust markers for breeders to use in marker-assisted selection.

University of Sydney component

The detection of race Ug99 of the wheat stem rust pathogen alarmed the international wheat community about its potential threat to global food security. The focus of this project was to broaden the genetic base of stem rust through identification and characterisation of diverse sources of resistance and development of linked molecular markers. We focused on characterisation of two pre-Green Revolution tall wheat genotypes from the Watkins collection; Aus28082 and Aus28230, identified to carry adult plant resistance against Ug99. Recombinant inbred populations (RILs) from Aus28082/Yitpi and Aus28230/Yitpi crosses were developed and screened in Australia, India and Kenya. The stem rust resistance genes carried by Aus28082 and Aus28230 were located in chromosomes 2B and 5D, respectively, and flanking markers were identified for their marker assisted selection in breeding programs. Aus28082 and Aus28230 and the stem rust susceptible parent Yitpi also carry adult plant resistance to stripe rust and leaf rust. Aus28082/Yitpi and Aus28230/Yitpi RIL populations were screened in Australia and Kenya against stripe rust and leaf rust. QTL analysis showed genetic association of resistance to stem rust and leaf rust on chromosome 2B of Aus28082. One QTL for stripe rust each, on chromosomes 3A and 5A from Aus28082 and Aus28230, was identified. An additional stripe rust resistance QTL on chromosome 3D was contributed by Yitpi. These populations are currently being screened in India for stripe rust and leaf rust under the coordinating project “Molecular marker technologies for faster wheat breeding in India – Phase 2” (CIM-2013-009) to assess the effective of these QTL under Indian conditions. Overall, two new sources of resistance to stem rust were identified.

Significance and application of the results

Stem rust resistance carried by Aus28230 in chromosome 5D is unique and has not been reported in any other study. The involvement of chromosome 2B in conditioning Adult Plant Resistance (APR) to stem rust has been observed in an association mapping study from the USA in addition to its detection in Aus28082 in this study. The APR to stem rust carried by Aus28082 showed association with APR to leaf rust as well. Leaf rust response data generated by Indian collaborators on Aus28082/Yitpi RIL population will confirm this genetic association based on Australian and Kenyan data. Screening of both RIL populations against Indian pathotypes of the stripe rust pathogen will confirm broad effectiveness of APR to stripe rust. APR genes (stem rust, leaf rust and stripe rust) identified in this study represent a significant addition to the existing sources of APR to wheat rusts.

Deployment of APR to rust diseases in commercial cultivars is preferred over All Stage Resistance (ASR) for its durability. A majority of the formally named genes belong to the ASR category and only five APR genes (Sr2, Sr55, Sr56, Sr57 and Sr58) have been named. Characterisation of two new loci in this project is a significant addition to the list of APR genes. Although analysis of APR to stripe rust and leaf rust was not the objective of this project, identification of unique loci for APR to stripe rust in Aus28082, Aus28230 and Yitpi and APR to leaf rust in Aus28082 provided valuable sources of resistance to combat these diseases as well. Transfer of APR from Aus28082 and Aus28230 to two leading Australian and Indian wheat cultivars each will be performed through marker assisted selection as part of the PhD of Deepak Baranwal (from Bihar Agricultural University, Sabour in Eastern India), a new John Allwright Fellow. Rust resistance genes Yr47, Lr52, Sr22, Sr26 and Sr50 were backcrossed into two Australian and two Indian wheat cultivars. Yr47, Lr52 and Sr50 (and 2 additional genes Yr51, Yr57, see below in Capacity Building) are not present in any modern cultivars. This activity therefore also fulfilled the objective of broadening the genetic base for rust resistance in Australian and Indian wheat breeding programs. Markers closely linked with genes have been published and are available for marker assisted selection. Triple rust resistant germplasm will be distributed to the Australian wheat breeding companies through the germplasm enhancement section of the Australian Cereal Rust Control Program and to the collaborating Indian programs through ACIAR-ICAR project CIM2013-009. Wheat growers from both nations will benefit from the release of triple rust resistant cultivars.

Capacity building

Mr Mandeep Randhawa (John Allwright Fellow in the Faster Wheat Breeding Project) worked on wheat rusts and completed his Ph.D. degree. He characterised two new stripe rust resistance genes from a wheat landrace Aus27858 and formally named them Yr51 and Yr57.

Two young scientists, one each from Shimla and Wellington rust laboratories, were trained in rust phenotyping, marker assisted selection and molecular fingerprinting of rust pathogens.

Conclusion

The project achieved its objectives, new rust resistance genes were characterized and linked markers identified. These project outputs have already been adopted by the on-going project CIM2013-009 and wheat breeders in India and Australia have accessed these materials for onwards development.

3 Background

A major goal in wheat improvement programs in India and Australia is to safeguard wheat production with genetic resistance against the three rust species, stem rust (*Puccinia graminis*), stripe rust (*Puccinia striiformis*) and leaf rust (*Puccinia triticina*). Research and development priorities continue to be directed at wheat resistance genes that are not only effective, but remain durable in spite of constantly evolving plant pathogen populations. As wheat accounts for most cereal production in both countries, it is further imperative that wheat in particular has more effective disease resistance. The emergence of a potentially devastating wheat stem rust race, Ug99 or TTKSK has threatened global wheat production and has raised biosecurity concerns for the wheat crop in India and Australia in the event of an introduction of Ug99 or other related races. Australia stands to gain from the experience in India which is closer to the predicted geographic path for spread of this group of stem rust races. In the absence of Ug99, India and Australia will still benefit from joint rust evaluations conducted in both countries to identify resistance genes effective against a broad range of pathotypes.

4 Objectives

Aim: The project aims to develop more durable resistance to stem rust through the combination of diverse sources of adult plant resistance and seedling resistance genes effective against Ug99 and its derivatives.

Objectives:

To identify potentially new genetic diversity for adult plant stem rust resistance to be deployed against Ug99 and derivative races

To develop and validate simple and robust molecular markers linked to genes effective against Ug99 and derivative races and to assist with the implementation of markers in breeding programs to produce rust resistant wheat cultivars

5 Methodology

CSIRO

A. Seedling and Adult Plant Resistance (APR) gene characterisation.

Combinations of three or more stem rust APR genes are required to achieve commercially acceptable levels of resistance. Of the 46 characterised and catalogued genes in wheat, only Sr2 belongs to the adult plant resistance (APR) category. Other less defined APR genes are present in cultivars such as the North American cultivars Chris, Thatcher and the Swiss winter wheat Forno that interact with the leaf and stripe rust resistance gene Lr34/Yr18 by conferring field resistance to stem rust. Other APR genes have been reported in the cultivar Pavon, Kingbird, Kiritati (being targeted for mapping in the Cornell DRRW) and also the Swiss winter wheat Arina (Sr56).

One main objective of this project was to characterise adult plant stem rust resistance in Thatcher wheat. 'Thatcher' is an old North American cultivar that carries complex resistance to stem rust that is further enhanced in the presence of the multi-pathogen resistance gene *Lr34*. Previously a RIL population was developed by Jim Kolmer USDA by crossing Thatcher +Lr34 (RL6058) to a related line that lacks stem rust resistance and Lr34 (RL6071). The population was evaluated in Australia for stem rust resistance in 2008 and stem rust susceptible progeny RL90 which retained Lr34 was selected to generate a new F4:F5 mapping family (94 RILs) that was used for mapping of stem rust resistance under field conditions in Kenya, USA and Canada. This population was also scored for race-specific Sr12 gene in seedlings using Canadian stem rust race.

RL90 (Sr-, Lr34+) x RL6058 (Sr+, Lr34+)

To study interactions between stem rust resistance with Lr34, another population was developed that was derived from a cross between RL90 and a Thatcher derivative that lacked Lr34. Using markers we selected homozygous lines which were fixed for presence/absence of stem rust resistance and Lr34. These lines were phenotyped in F3 generation at the seedling stage using Australian stem rust race. Early stem rust infection was assessed using a sensitive chitin assay that determines the fungal biomass in the leaf tissue before macroscopic symptoms become visible.

RL90 (Sr-, Lr34+) x RL6077 (Sr+, Lr34-)

Two additional populations were evaluated in the field in Kenya and Canada for stem rust resistance: 1. 120 RILs derived from cross between Chinese Spring and Tc+Lr34 and 2. 180 RILs developed from crossing Kenyon (Tc derivative) with susceptible breeding line 8615MN-2137. Markers identified in RL population were evaluated in these populations to confirm the overlap of QTL region identified on chromosome 3B.

B: Molecular marker analysis

Bulk segregant and selective genotyping approaches were used to genotype lines that were resistant and susceptible in all three field environments. SNP arrays were used to identify linked markers that were later converted into robust KASP assays for fine scale mapping. Tightly linked markers were evaluated in diverse wheat germplasm to assess allele frequencies and utility for breeders. Marker assisted selection was also used to transfer stem rust resistance into susceptible, adapted backgrounds for further field evaluation of resistance in unrelated backgrounds.

University of Sydney

Population development

To identify more APR genes, a historic collection of wheat cultivars/landraces (Watkin collection) was screened under field conditions over three years. These genotypes were screened in the seedling stage to identify seedling susceptible and adult plant resistant genotypes. Genotyping of cultivars carrying APR to stem rust with a marker closely linked with *Sr2*, indicated that resistance is likely to be based on genes different than *Sr2*. Wheat landraces Aus28082 and Aus28230 carrying adult plant resistance (APR) to stem rust were crossed with stem rust susceptible cultivar Yitpi and F6 recombinant inbred line (RIL) populations were generated. F6 populations (103 Aus28082/Yitpi and 120 Aus28230 RILs) were tested in Australia, India and Kenya. Due to segregation for late maturity some lines did not flower in Kenya and India. Experimental plots were inoculated using Australian pathotypes 98-1,2,3,5,6,7 (culture 580) and 34-1,2,7+Sr38 (565), Indian pathotype 40A and Kenyan pathotype Ug99+Sr24. Standard protocols for screening were followed. Aus28082/Yitpi and Aus28230/Yitpi RIL populations showed segregation for stripe rust and leaf rust in Australia and Kenya and hence were scored for variation for these diseases as well.

QTL analysis

Aus28082/Yitpi and Aus28230/Yitpi RIL populations were genotyped using the iSelect 90K Infinium assay and QTL analyses for adult plant responses for three rust diseases were performed using standard procedures. Flanking SNPs were converted into KASP assays for marker assisted selection.

John Allwright Fellow – Mandeep Randhawa

Aus27858 was crossed with a stripe rust susceptible cultivar Westonia to develop a F3 population for genetic analysis. Seedling stripe rust tests on F3 population showed digenic segregation for seedling stripe rust response. One hundred seedlings from two F3 lines segregating at a single locus for different infection types were grown and two F6 RILs were developed. Bulked segregant analysis on these RIL populations was performed using DArT markers. The target genomic regions were enriched using various genomic resources. Markers linked with resistance genes were validated on a set of Indian and Australian cultivars. He also conducted screening of international germplasm both under the greenhouse and field conditions.

6 Achievements against activities and outputs/milestones

Objective 1: To identify potentially new genetic diversity for adult plant stem rust resistance to be deployed against Ug99 and derivative races

no.	activity	outputs/ milestones	due date of output/ milestone	risks / assumptions	applications of outputs
1.1	Grow F3 families from two APR sources in Watkin collection for single gene family development	Monogenically segregating families identified	Nov. 30, 2009	Failure to create good epidemic under field conditions	Single gene families available for generation of monogenically segregating populations. Rust results available for initial marker screen and/or bulked segregant analyses.
		Monogenically segregating populations developed (Genetically it will be equivalent to F3)	May 30, 2010	Mechanical failures including light and temperature controls in the greenhouse.	F3 equivalent generation produced
		Next generation of single seed descent populations produced and the previous generation screened for stem rust response variation under field conditions	Nov 30, 2010	Mechanical failures including light and temperature controls in the greenhouse. Failure to create good epidemic under field conditions	F4 generation produced and rust results on F3 results from monogenically segregating F3s available for marker work
		Next generation of single seed descent populations produced.	March 30, 2011	Mechanical failures including light and temperature controls in the greenhouse.	F5 generation produced
		F5 monogenically segregating populations grown for bulking up	Aug 30, 2011	Mechanical failures including light and temperature controls in the greenhouse.	F6 Generation produced
		F5 populations screened under field conditions for stem rust response variation	Dec 20, 2011	Failure to create good epidemic	Stem rust results available for marker work
		Seed of F6 monogenically sgregating populations to India and and Kenya sent	Oct.30, 2011	Delayed receival of MTAs and import permits	Stem rust response results verified in India and Kenya

		Stem rust segregation among monogenically segregation populations noted in Australia, Inida and Kenya and data compiled	Dec. 30, 2012	Failure to create good epidemic	Stem rust results available for finishing the marker work
		Confirmation of monogenic segregation among isolated gene families and multiplication of seed	Dec. 23, 2013	Failure to create good epidemic	Stem rust response data will be available for mapping
		Screen F6 families derived from crossess of landarces AUS28082 and AUS28230 with Yitpi at Cobbitty, Wellington, Njoro in 2013	Dec.23, 2013	Failure to create good epidemic	Stem rust response data will be available for mapping
		Repeat screening of F6 families from both crosses and confirmed single gene mapping families at Cobbitty, Wellington, Njoro in 2014	Dec. 23, 2014	Failure to create good epidemic	Stem rust response data will be available for mapping
1.2	Develop single gene families from RL6058 and Chris in susceptible background	Phenotype for APR stem rust resistance determined for BC1 families in the field.	Dec 2010	One backcross may not be sufficient to separate multiple resistance genes	
		Phenotype mapping families for stem rust resistance in the field.	Dec 2011	Single gene families will be fully classified into either susceptible or resistant lines	Single gene families used for marker development
		Phenotype putative single gene mapping families in the field	Dec 2012	Assume good correlation of rust scores between field sites	Single gene mapping families confirmed
		Phenotype putative single gene mapping families at seedling stage	Sept 2013	Relying on collaborators to carry out screening	Segregation of seedling resistance determined
		Phenotype single gene families in the field	Dec 2013	Requiring irrigation and spreader rows for good infection	Rust scores confirmed in Cobbitty and Kenya
		Phenotype single gene families in the field	Dec 2014	Requiring irrigation and spreader rows for good infection	Rust scores confirmed in Cobbitty and Kenya

PC = partner country, A = Australia

Objective 2: To develop and validate simple and robust molecular markers linked to genes effective against Ug99 and derivative races and to assist with the

implementation of markers in breeding programs to produce rust resistant wheat cultivars

no.	activity	outputs/ milestones	due date of output/ milestone	risks / assumptions	applications of outputs
2.1	Molecular marker development for Ug99 effective seedling resistance genes	Validated molecular marker for Sr22 in Australian and Indian cultivar backgrounds	June 2009	Marker may not differentiate all the recurrent parental cultivars targeted for Sr22 introgression	Combine Sr22 with other genes in pyramiding.
		Tightly linked Sr13 marker diagnostic in a reference set of Indian and Australian cultivars	December 2010	Marker may not differentiate all the recurrent parental cultivars used in Sr13 NIL	Combine Sr13 with other genes in pyramiding.
2.2	Chromosomal regions and marker development for new APR genes	Chromosomal regions identified that carry new APR Sr genes	Feb 2011	Chromosomal regions may overlap with other activities in Cornell DRRW project	
		Validated molecular markers in Australian and Indian background for new APR Sr genes	Dec 2012	Markers may not be informative in all genetic backgrounds	Combine new APR genes with other effective Sr genes using markers
		Genotype single gene families (Thatcher - derived) with SNP based markers	Oct 2013	Current markers may not be tightly linked	Linkage estimate between markers and QTL regions
		BSA using rust scores from 2012/13	Feb 2014	90 K array may not generate additional markers	Additional tightly linked markers identified
		Genotype single gene families with SNP based markers	July 2014		Fine scale mapping of resistance gene(s)
		Development of robust, tightly linked markers	Dec 2014		Robust markers tested in diverse germplasm and passed onto CIM 2005-20
2.3	Joint ACIAR-Cornell DRRW marker development and validation of new APR genes	Chromosomal location of new APR gene from Pavon/Hartog	April 2011	Markers may not be informative between Hartog, Sei82 and Genaro81	Determine the additive effect of Sr2 and additional APR gene(s) from Pavon/Hartog

2.4	Genomic location of stem rust resistance and identification of closely linked markers - Watkins landraces	DNA from F6 mapping populations derived from crosses of landraces AUS28082 and AUS28230 will be sent for 90k SNP/GBS genotyping	May 2013	Markers may not be tightly linked	Stem rust response linked markers identified
		Crosses of these two sources will be made with two Indian and two Australian stem rust susceptible cultivars	October 2013	Hybrid dwarfism and/or progressive necrosis may affect	F1s generated
		Selective genotyping of single gene mapping families/original F7 populations from two crosses	February 2014	Lack of polymorphism	Closely linked markers identified
		Backcross F1s to recurrent parent	February 2014	Greenhouse air-conditioning failure	Backcross F1s generated
		Backcross F1s grown in field and stem rust selection performed	Dec 2014	Failure to create good rust epidemic	Backcross F2 seed harvested

PC = partner country, A = Australia

7 Key results and discussion

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Results

The purpose of this study was to examine adult-plant resistance (APR) in 'Thatcher' and look for genetic interactions with *Lr34*. A RIL population fixed for *Lr34* was produced from the cross RL90/RL6058 (RL6058 = Thatcher+Lr34) and was tested for stem rust resistance in field nurseries in Canada, USA, and Kenya. Highly resistant and highly susceptible RILs were used for bulked segregant analysis (BSA) to find SNP markers associated with reduced stem rust severity. Identified SNPs were applied to the entire RIL population and a major QTL was identified on chromosome 3BL near the centromere in all environments (Fig1, Table1). Seedling testing showed that *Sr12* mapped to the same region as the QTL for APR. The results indicate that the genetic interval carrying stem rust resistance which was observed under field conditions in USA, Kenya and Canada overlapped with the map position of *Sr12* as determined by seedling tests.

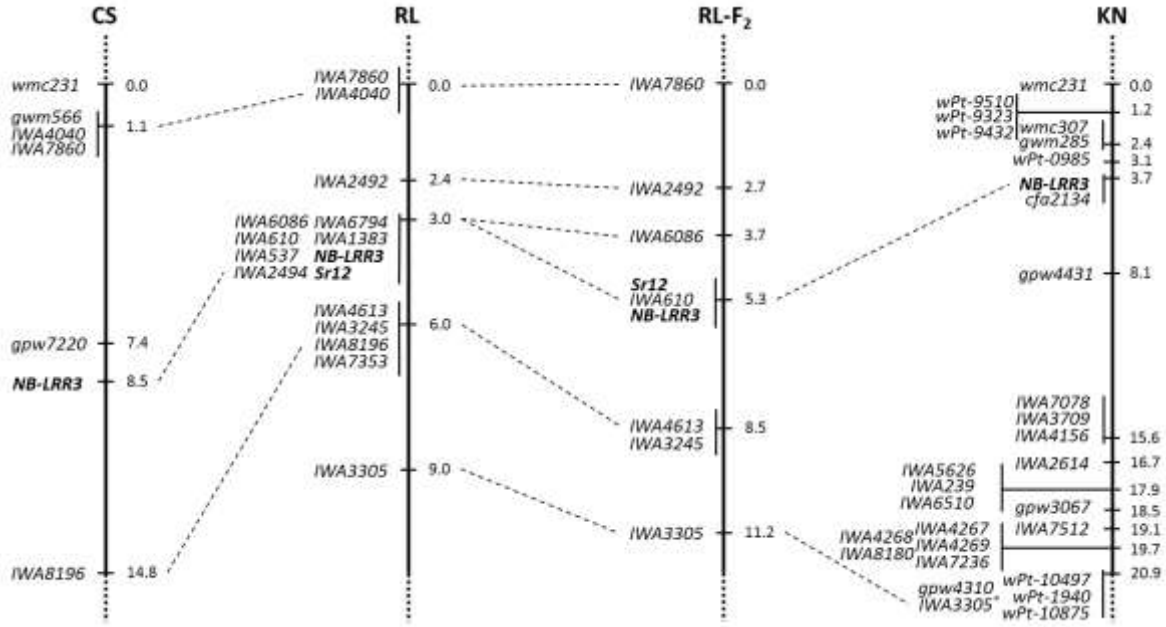

Fig 1. Genetic maps of the proximal region of chromosome 3BL in the Chinese Spring/RL6058 (CS) DH, RL90/RL6058 (RL) RIL, RL90/RL6058 F_2-equivalent (RL-F_2), and Kenyon/8615MN-2137 (KN) RIL populations. Map positions are in centi-Morgans.

Table 1. Statistics for QTL analysis (interval mapping) of stem rust severity in the RL, CS, and KN populations.

Population	Environment	Position (cM)[a]	LOD[b]	PVE(%)[c]	Add[d]
RL	Canada 2014	3.0	28.98	83.93	-24.31
RL	Canada 2015	2.4	19.17	70.16	-21.11
RL	Kenya 2013	3.6	14.14	56.26	-9.12

RL	USA 2013	3.5	20.29	69.94	-14.40
CS	Canada 2014	7.0	12.09	33.68	-13.19
KN	Kenya 2013	3.1	4.13	18.16	-7.88
KN	Kenya 2014	3.1	5.16	22.12	-9.30

[a] The "position" refers to the position on the genetic maps of chromosome arm 3BL for each population shown in Fig. 1.

[b] The significant LOD thresholds for the RL and CS populations were set at 3.0 as permutation analysis is inappropriate in the absence of a full genome map. However, if thresholds from a permutation test were used, the significance threshold would be less stringent. Permutation analysis (1000 permutations) was performed for the KN population and the significant LOD threshold was 3.4 for 2013 and 3.5 for 2014.[c] Percent variation explained for stem rust severity by the QTL on chromosome arm 3BL .[d] The additive effect that the resistant allele has on stem rust severity.

Using the chromosome 3B pseudomolecule of Chinese Spring, the SNP markers were physically mapped and sequences from the region carrying the resistance was searched for sequences with homology to members of the nucleotide binding- leucine rich repeat (NB-LRR) resistance gene family. SNP markers were developed for NB-LRR-like sequences and one, *NB-LRR3* co-segregated with *Sr12*. 'Chinese Spring' contained a partial NB-LRR gene in this region which may be homologous to a candidate gene for *Sr12* in RL6058.

Two additional populations, including Chinese Spring x RL6058 and Kenyon x 8615MN-2137 which lacked *Lr34* entirely, were tested in field nurseries. *NB-LRR3* mapped near the maximum LOD for reduction in stem rust severity in both populations (Fig1). Furthermore, *NB-LRR3* was added to a genetic map of chromosome 3B in another Thatcher derived population that was previously used to map *Sr12* (Rouse et al. 2014). In this population *NB-LRR3* also co-located with a major QTL for stem rust resistance that was observed under field conditions on chromosome 3B and co-segregated with race specific seedling resistance, which was presumably *Sr12*.

Previous reports showed that the combination of *Lr34* with additional genes from 'Thatcher' can contribute to enhanced stem rust resistance under field conditions (Gavin Vanegas et al.2008; Rouse et al. 2014). We have quantified possible interactions by examining the effect of *Lr34* on *Sr12* at the seedling stage. Homozygous sister lines from the RL90 x RL6077 population that represented four genotypic categories; null (neither *Lr34* nor *Sr12* were present), *Lr34*, *Sr12*, *Lr34+Sr12* were evaluated for stem rust infection The level of stem rust infection was determined by measuring chitin levels in leaf tissue at 10 DPI (Ayliffe et al. 2013). Single gene lines carrying either *Lr34* or *Sr12* showed a reduction in stem rust infection compared to null lines but lines with both genes reduced infection levels even further (Fig. 2). Macroscopic symptoms at 14 DPI also reflected these differences (data not shown). Lines carrying both genes were also more resistant than single gene lines when tested in the field in Minnesota in 2014 and in Canada in

2015 (data not shown). Under field conditions in our trials *Lr34* by itself did not contribute to stem rust resistance, indicating that stem rust resistance involving *Lr34* and *Sr12* is epistatic. We concluded that *Sr12* or a gene closely linked to *Sr12* was responsible for 'Thatcher'-derived APR in several environments and this resistance was enhanced in the presence of *Lr34*.

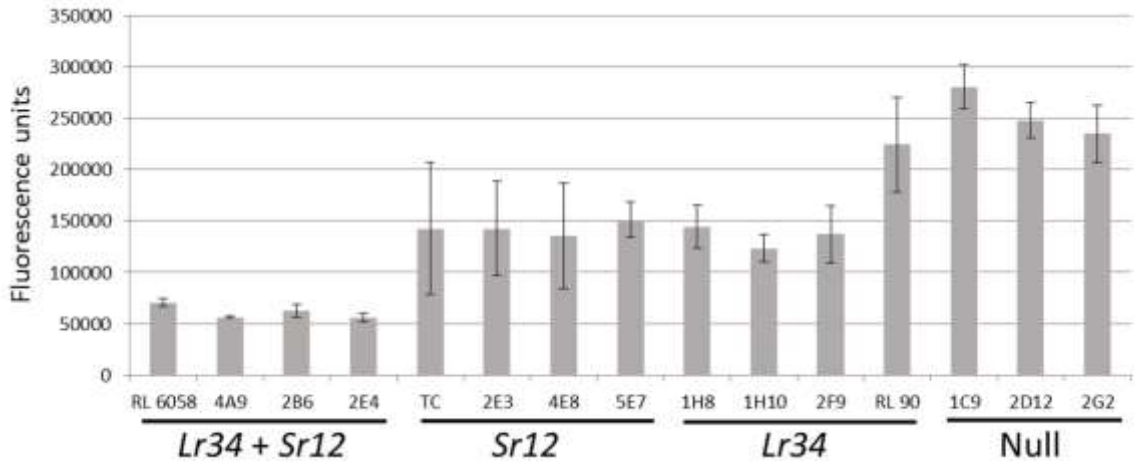

Fig 2. Amount of *Sr12*-avirulent *Pgt* race 98-1,2,(3),(5),6 infection at the seedling stage after 10 DPI in four genotypic classes of homozygous lines from the RL90/RL6077 population as determined by an assay measuring chitin abundance in the leaf tissue. TC represents 'Thatcher'.

To evaluate the usefulness of the *NB-LRR3* marker for marker-assisted selection, the allele frequency was determined in 'Thatcher' derivatives and historic North American germplasm with known *Sr12* status. In these lines, the marker genotype corresponded with the presence/absence of *Sr12* including for 'Iumillo' durum, the donor line for *Sr12*. We also tested the allele frequency of *NB-LRR3* and other linked SNP markers in 92 Australian cultivars of unknown *Sr12* status. The 'Thatcher' allele of *NB-LRR3* and *IWA610* was present in approximately 50% of lines tested. The *NB-LRR3* marker was tested on 97 wheat cultivars and lines developed in or introduced into Canada. In total, 50 lines carried the 'Thatcher' allele of *NB-LRR3* while 47 did not. The influence of 'Thatcher' in the genealogy of Canadian wheat is apparent in the results. Most of these cultivars that carry the Thatcher allele of *NB-LRR3* have Thatcher in their pedigrees.

Discussion

Stem rust resistance in the field co-located with the race-specific *Sr12* gene on chromosome arm 3BL in several Thatcher derived populations. The *NB-LRR3* marker which explained a large proportion of phenotypic variation for stem rust resistance in the field, co-segregated with *Sr12* in these populations. However, it is unknown if *Sr12* is responsible, in whole or in part, for the APR observed on chromosome arm 3BL. *Sr12* is ineffective at the seedling stage to field races in Kenya and is largely ineffective to North American races of *Pgt*. There are two possible explanations: APR and *Sr12* are controlled by independent genes on 3BL, and/or under field conditions *Sr12* can provide APR against races that are virulent in the seedling stage. Major genes are typically assessed

for effectiveness by seedling tests; however, relatively little emphasis is placed on their utility in “field resistance”. It appears that some defeated or partially defeated rust resistance genes may play a role in APR when combined with other genes.

A goal of this study was to identify and genetically map stem rust resistance in ‘Thatcher’ that is expressed or enhanced in the presence of *Lr34*. To test for interactions, sister lines were developed from a population that was segregating for *Lr34* and *Sr12* in the RL90/RL6077 population. Using these lines we showed that *Lr34* reduced stem rust infection at the seedling stage. Using a chitin assay to determine the fungal biomass provided a sensitive assay before symptoms became visible. Field tests of these lines showed that stem rust severity in each genotypic group showed agreement with both the seedling test for IT and fungal biomass. It is possible that *Sr12* may contribute APR by itself or through interactions with other genes such as Lr34. It is possible that these gene interactions are relatively common but remain poorly understood.

Stem rust resistance in ‘Thatcher’ has been studied extensively but the resistance was found to be complex and difficult to dissect (Knott 2000). Recent studies focused on studying gene interactions to explain components of resistance and identify linked markers. For example, one study identified resistance from ‘Thatcher’ located on chromosome arm 2BL that interacted with *Lr34* but did not report resistance on chromosome 3B (Gavin Vanegas et al. 2008; Kolmer et al. 2011). In another study analysing the stem rust resistance in ‘Thatcher’ four QTLs were identified (Rouse et al. 2014). Of these QTLs reported only the QTL on chromosome 3B was significant in all three field environments and it corresponded to the mapped location of *Sr12*. A QTL on 7DS corresponded to *Lr34*, which interacted with the 3B resistance by reducing rust severity in the field. The association between the *Sr12* locus and APR in the field is consistent with our findings.

This project brought together expertise of researchers who are currently working on Thatcher stem rust resistance including Colin Hiebert from AgCanada and Jim Kolmer and Matt Rouse from USDA Minnesota. The collaboration enabled the sharing of germplasm that allowed phenotyping of material in diverse environments and against wider range of pathotypes. We also exchanged marker information which allowed us to link the major QTL on 3B to a common marker across several populations. The research links are being maintained to dissect stem rust resistance in Thatcher further and to identify resistance genes that are part of the durable resistance complex.

University of Sydney

Results

The project was focused on characterisation of adult plant resistance to stem rust in landraces Aus28082 and Aus28230. Aus28082/Yitpi and Aus28230/Yitpi RIL populations were screened at two experimental sites of the University of Plant Breeding Institute, Cobbitty, Indian Agricultural Research Institute Regional Research Station, Wellington and Kenya Agricultural & Livestock Research Organisation, Njoro. Both RIL populations were genotyped using iSelect 90K Infinium assays.

QTL analysis identified the involvement of chromosome 2B (*QSr.sun-2B; Sr993*) in controlling stem rust resistance carried by Aus28082. Markers *IBW25129* and *IBW80340*

were the closest in all datasets sets. *QSr.sun-2B* explained 14 to 18% phenotypic variation. QTL, *QSr.sun-5D* (*Sr1141*), controlled stem rust resistance in Aus28230 and it peaked at the marker *IBW34532* in all datasets. The phenotypic variation explained across datasets varied from 25% to 49%.

Phenotypic data for both populations was compared with allelic segregation at the most closely linked marker loci. RILs carrying the Aus28082 and Aus28230 alleles showed stem rust response scores of 5 or below, whereas RILs carrying Yitpi allele exhibited stem rust responses varying between 6 and 7. Yitpi was scored 6 or 7 in all experiments. Data were converted to genotypes with RILs exhibiting responses of 5 or below as 'A' and 6 and above as 'B' and incorporated in the respective maps. Stem rust resistance carried by Aus28082 mapped on chromosome 2B and the resistance locus was flanked by markers *IBW25129* and *IBW80340* at genetic distance 2.7 and 3.3 cM, respectively (Figure 1a). Similarly, stem rust resistance carried by Aus28230 mapped to chromosome 5D and it was flanked by markers *IBW34532* and *IBW77287* at 1.8 cM and 3.3 cM, respectively. KASP assay have been designed for these markers to test their efficacy in marker assisted selection (MAS) of these QTL/genes. Mr Deepak Barnawal, John Allwright Fellow 2016, will conduct pyramiding of these genes with other APR genes (*Sr2*, *Sr56*, *Sr57*, *Sr58*).

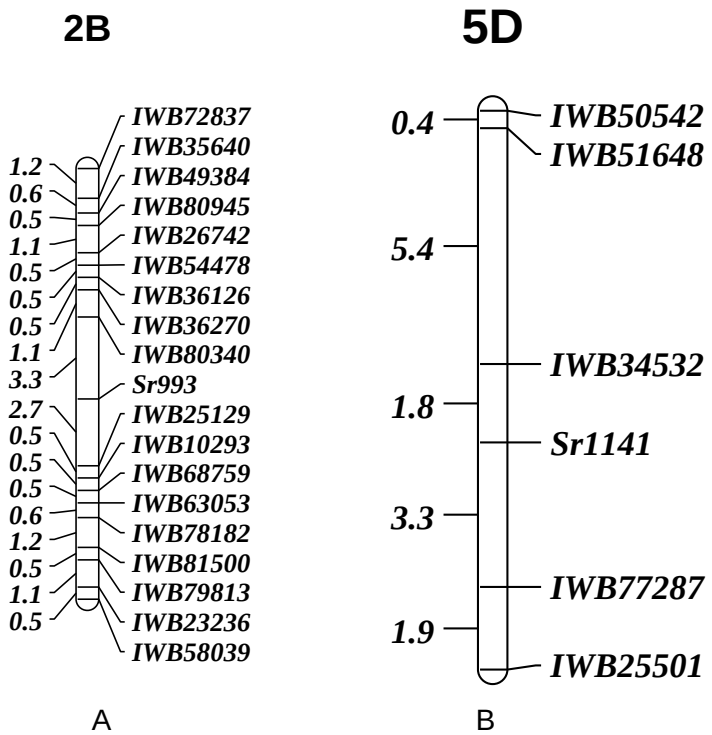

Fig.1: Genetic maps showing location of stem rust resistance carried by A) Aus28082 and B) Aus28230

In addition stripe rust QTL on chromosomes 3A of Aus28082, 5A of Aus28230 and 3D of Yitpi were identified. *QSr.sun-2B* also conditioned adult plant resistance to leaf rust. Overall, APR genes for resistance to three rust pathogens were identified among Aus28082/Yitpi and Aus28230/Yitpi RIL populations.

Drs Om Prakash Gangwar (Shimla) and Vikas Venu Kumaran (Wellington) were trained in rust phenotyping, MAS and fingerprinting of rust pathogens. Both scientists attended BGRI Technical Workshop and International Wheat Conference in Sydney. Dr Mandeep

Randhawa, John Allwright Fellow, characterised and named two new stripe rust resistance loci (*Yr51* and *Yr57*) from another landrace Aus27858 and developed markers linked with these resistance genes. Sources carrying these resistance genes have been provided to Indian and Australian breeders through CIM2013-009.

India

A total of 1189 lines were screened under natural epiphytotic conditions during the last two years at the IARI-RS, Wellington (three rusts), Dhaulakuan and Malan (stripe rust) and Karnal (stripe rust). A set of 413 lines were tested at the seedling stage against rust diseases at the IIWBR-RS, Shimla (stripe rust - 11 pathotypes, leaf rust - 10 pathotypes and stem rust - 7 pathotypes) during 2013-14. Eleven and nine lines carried resistance to stripe rust and leaf rust, respectively, and four lines were resistant to all three rust diseases. These lines have been crossed with PBW343 and WL711 for genetic analysis.

Discussion

This project identified two new genes for adult plant resistance to stem rust from two wheat landraces. One of the QTL (*QSr.sun-2B*) also conferred adult plant resistance to leaf rust. This stem rust and leaf rust association was also observed in durum landrace (Bansal and Bariana unpublished). Seedling stem rust resistance gene *Sr9b* is located in the same region of chromosome 2B (McIntosh et al. 1995) as *QSr.sun-2B*. Comparative rust tests with *Sr9b*-avirulent and virulent pathotypes showed that *QSr.sun-2B* and *Sr9b* are different. *QSr.sun-5D* maps in the region where *Sr30* is located (McIntosh et al. 1995). *Sr30* has been reported to be ineffective against Ug99 (Pretorius et al. 2000). Crosses have been made to study relationship of these loci. SNP markers flanking both *QSr.sun-2B* and *QSr.sun-5D* have been identified and are available for use in breeding programs in Australia through the germplasm delivery pipeline of the Australian Cereal Rust Control Program and in India through the coordinating project “Molecular marker technologies for faster wheat breeding in India – Phase 2” (CIM-2013-009).

This project strengthened ‘the global rust workers network’ that existed under the Australian Cereal Rust Control program. Mentoring of young scientists in India and in rust workshops attended by project scientists (Bariana and Bansal) is expected to have impact during the next decade. The mentoring of young Indian scientists is continuing through the “Molecular marker technologies for faster wheat breeding in India – Phase 2” project (CIM-2013-009).

8 Impacts

8.1 Scientific impacts – now and in 5 years

CSIRO

We mapped adult plant stem rust resistance gene in Tc on chromosome 3B that is broadly effective against field races in Kenya, US and Canada. We have shown that resistance on 3B is enhanced by Lr34 and is likely to contribute to durable resistance. Thatcher carries complex but durable stem rust resistance. Dissecting components of resistance and tagging genes with markers will provide breeders with additional options to combat the threat. Robust KASP assay was developed for the APR gene on chromosome 3B that is now available for marker-assisted selection. A candidate gene was identified which requires proof of function.

The marker developed in this project was used to transfer Sr gene on 3B into adapted, susceptible Indian lines PBW343 and Kharchia. BC1 derived lines will be available for field testing next year. These follow up experiments will provide insight into two critical questions: 1.) Does Sr gene on 3B confer stem rust resistance in other backgrounds and 2.) is the gene enhanced in the presence of Lr34.

Our results raised the possibility that Sr12 confers field resistance to races which are virulent at the seedling stage. This raises an important question about the value of so called 'defeated' R genes in breeding. We plan to address this question by testing mutants which lost Sr12 seedling resistance in the field next year.

University of Sydney

Five new APR loci (*QSr.sun-2B/QLr.sun-2B*, *QSr.sun-5D*, *QYr.sun-3A*, *QYr.sun-3D* and *QYr.sun-5A*) were identified in this project and markers flanking *QSr.sun-2B/QLr.sun-2B*, *QSr.sun-5D* were identified. These markers are now available for their pyramiding with other molecularly-tagged rust resistance genes. The stacking of these new loci with the existing five APR genes *Sr2*, *Sr55*/*Lr67*/*Yr46*, *Sr56*, *Sr57/Lr34/Yr18* and *Sr58/Lr46/Yr29* and currently effective all stage resistance genes will contribute to durable rust control in both nations and elsewhere. All stage stripe rust resistance loci *Yr51* and *Yr57* identified by the John Allwright Fellow associated with this project are effective in both nations. Closely linked markers and improved germplasm carrying these genes is being used in breeding programs and is expected to have significant impact. Training of young scientists will ensure effective delivery of scientific outputs in India. Dr Mandeep Randhawa, John Allwright Fellow has joined the Global Wheat Program of CIMMYT, Mexico and will use the knowledge and germplasm developed in the project to develop triple rust resistant wheat varieties.

8.2 Capacity impacts – now and in 5 years

Drs Om Prakash Gangwar (Shimla) and Vikas Venu Kumaran (Wellington) were trained in classical and molecular aspects of rust research. Their training will facilitate effective delivery of rust resistant germplasm to Indian wheat breeders. In addition, Dr Hanif Khan from the Shimla laboratory, was trained under the Endeavour Post-Doctoral Fellowship program. The appointment of Dr Mandeep Randhawa in the Global Wheat Improvement Program will deliver benefits to the broader community.

Harbans Bariana delivered a guest lecture on Indo-Australian collaboration to breed rust resistant wheat cultivars at the launch of Gold Medal for M.Sc. Plant Pathology graduates at Ludhiana. Bansal and Bariana organised a workshop on marker assisted selection of rust resistance in wheat. This workshop was attended by two young scientists from India and two from Uruguay. The aim of the workshop was to build a 'Tri-Continental Team' to Combat Rust Diseases.

8.3 Community impacts – now and in 5 years

8.3.1 Economic impacts

Rust diseases are among the top biotic constraints to wheat production in both nations. Deployment of marker-tagged diverse sources of rust resistance identified in this project is a key component of the ongoing maintenance and improvement of the wheat varieties used in India and Australia. Resistant varieties reduce risks, reduce costs and improve resilience for the farmers deploying them. They contribute to farm gate returns in both nations.

8.3.2 Social impacts

Too difficult to assess

8.3.3 Environmental impacts

Deployment of rust resistant wheat cultivars will reduce the dependency on fungicides to control these diseases. Reducing fungicide use is limiting the limiting the selection pressure on the fungal pathogen, and is also limiting the potential environmental impact of these chemicals.

8.4 Communication and dissemination activities

CSIRO

Oral presentations in international meetings:

Spielmeyer W, Hiebert C, Mago R, Tabe, L, Kolmer J Durable but complex: stem rust resistance from emmer wheat, International Cereal Rust and Mildew Conference, Copenhagen Denmark, Sept 2015

Hiebert C, Kolmer J, McCartney C, Fetch T, Choulet F, Bariana H, Spielmeyer W (2015) Mapping stem rust resistance on chromosome 3BL in Thatcher that interacts with Lr34. Plant and Animal Genome Conference XXIII, January 10-14, 2014, San Diego, CA

University of Sydney

Harbans Bariana attended all annual ACIAR-ICAR science meetings in India during the project tenure. He also delivered a lecture on Indo-Australian collaboration at the Punjab Agricultural University, Ludhiana. Project related results have been presented at various national and international meetings (e.g. Borlaug Global Rust Initiative (BGRI) Technical Workshop – annual event, Wheat Breeders Assembly, International Wheat Conference etc.)

Randhawa MS, Bansal UK, Bains NS, Trethowan RT, Bariana HS (2015) Identification of markers closely linked with *Yr51* and *Yr57* and their implementation in wheat improvement. International Wheat Conference, Sydney

Randhawa MS, Bansal UK, Bariana HS (2014) *Yr57* – A new locus for stripe rust resistance in wheat. BGRI Technical Workshop, Mexico

Randhawa MS, Bansal UK, Valarik, M, Bariana HS (2013) Closely linked markers for *Yr51* – From discovery to deployment. BGRI Technical Workshop, USA

9 Conclusions and recommendations

9.1 Conclusions

CSIRO

The goal was to improve our understanding why Thatcher-*Lr34* (RL6058) is significantly more resistant to stem rust than 'Thatcher'. Our study identified a proximal region on chromosome 3BL as the most significant factor for stem rust resistance in populations which were fixed for *Lr34*. Unexpectedly, this region was also responsible for the resistance to race Ug99 in Kenya identified in a population which lacked *Lr34*. Further analysis showed that *Sr12* is either responsible or closely linked to the field resistance observed in Kenya, the USA, and Canada. Seedling tests demonstrated that stem rust resistance on 3BL is enhanced in the presence of *Lr34*. Given these results, selection of *Sr12* region could provide a component of durable stem rust resistance in breeding programs and the *NB-LRR3* marker is a useful tool to assist in the selection process. In the meantime, work continues to identify the *Sr12* resistance gene through mutagenesis and the sequencing of NB-LRR gene sequences from mutants. The work is expected to generate a gene-based marker for *Sr12* and shed light on the relationship of *Sr12* and the associated APR.

University of Sydney

The aim was to identify new sources of adult plant stem rust resistance from Watkins landraces. The project characterised two new sources (*QSr.sun-2BL* and *QSr.sun-5DL*) of adult plant stem rust resistance carried by wheat landraces. Markers were identified for marker assisted selection of these loci in breeding programs. *QSr.sun-2BL* appears to confer adult plant resistance to leaf rust as well. In addition, one genomic region each from two landraces and the Australian cultivar Yitpi was observed to condition adult plant resistance to stripe rust under Australian and Kenyan conditions. Markers linked with two new stripe rust resistance loci *Yr51* and *Yr57* were also developed under the John Allwright Fellowship (Dr Mandeep Randhawa) program.

Two scientists from India were trained in greenhouse and field screening aspects of rust research. They were also trained in marker assisted selection for rust resistance in wheat and molecular finger printing of wheat rust pathogens at Cobbitty. Mentoring of junior scientists and postgraduate students at ACIAR-ICAR science meetings in India was conducted.

Overall, the project delivered two new marker-tagged sources each of stem rust and stripe rust resistance for use in Indo-Australian project "Molecular marker technologies for faster wheat breeding in India – Phase 2" project (CIM-2013-009).

9.2 Recommendations

CSIRO

A robust marker is now available to transfer the 3B gene into adapted Indian and Australian germplasm as part of the ongoing Marker implementation project. We

demonstrated synergistic interaction with Lr34, both these genes can now be combined using the previously published gene based marker.

Future opportunities: The breeding value of genes should be re-assessed based on the finding that a 'defeated' race-specific R gene may contribute to broadly effective field resistance. The effect of all known race-specific resistance genes in Thatcher should be studied by developing knock out mutants and testing single and double mutants in the field against stem rust. This will inform us if so called 'defeated' genes contribute to field resistance and estimate their effect. At the same time we will gain better understanding of other components of durable stem rust resistance in Thatcher and develop markers that will assist breeders to combine effective resistance genes in future cultivars.

University of Sydney

Transfer of rust resistance loci identified in this project to Australian and Indian wheat backgrounds will increase diversity for rust resistance. We recommend this germplasm development to be part of Mr Deepak Barnwal's (John Allwright Fellow from the Bihar Agricultural University, India) Ph.D. project. The pyramiding of these loci with previously characterised APR genes (*Lr34/Yr18/Sr57*, *Lr46/Yr29/Sr57*, *Lr67/Yr46/Sr55*, *Sr2*, *Sr56*) should be performed to achieve durable control of rust diseases of wheat. We recommend that these new combinations of rust genes be used extensively across CIM2013-009 to enhance the germplasm in partner centres and in other locations requiring high levels of rust resistance.

Future opportunities: Detailed characterisation of adult plant leaf rust and stripe rust resistance carried by Aus28082, Aus29230 and Yitpi should continue.

10 References

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Ayliffe M, Periyannan SK, Feechan A, Dry I, Schumann U, Wang M-B, Pryor A, Lagudah E (2013) A simple method for comparing fungal biomass in infected plant tissues. Mol Plant Microbe Interact 26:658-667

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10.2 List of publications produced by project

Colin Hiebert, Jordan Briggs, Jim Kolmer, Curt McCartney, Tom Fetch, Harbans Bariana, Fred Choulet, Matthew N. Rouse, Wolfgang Spielmeyer (2016) Field stem rust resistance in wheat co-locates with resistance gene *Sr12* on chromosome 3B in 'Thatcher' wheat. Submitted to PlosOne

Hiebert C, Spielmeyer W, McCartney C, Kassa M, Fetch T, You F, Menzies J, Humphreys G, McCallum B (2015) Stem Rust Resistance: Two Approaches. In: Ogihara Y, Takumi S, Handa H (eds) Advances in wheat genetics: From genome to field. Springer, Tokyo, Japan, pp 183-191

Randhawa MS, Bariana HS, Mago R, Bansal UK (2015) Mapping of a new stripe rust resistance locus in chromosome 3BS of wheat. Molecular Breeding 35: 65

Randhawa MS, Bansal UK, Valárik M, Klocová B, Doležel J, Bariana HS (2014) Molecular mapping of stripe rust resistance gene *Yr51* in chromosome 4AL of wheat. Theor Appl Genet 127:317–324