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1 Acknowledgments

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Fig. 1: Project inception meeting at Coral Dubai Deira Hotel, Dubai (27–28 February 2017)

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2 Executive summary

Disease epidemics resulting from new virulent races of pathogens and climate change are making it hard to increase wheat production, especially in developing countries. Stripe/yellow rust (WYR) remains one of the most damaging diseases of wheat world-wide, causing devastating epidemics in south Asia (SA) and in the highlands of east Africa (EA). One way to future-proof global wheat production against rust diseases is to utilize race (pathotype) non-specific durable adult plant resistance (APR). To mitigate the effects of WYR on wheat production in SA and EA, the University of Sydney collaborated with key cereal pathology and breeding personnel from EIAR (Ethiopia), IIWBR (India), NARC (Nepal) and PARC (Pakistan). Goals were set to characterise wheat germplasm for resistance to WYR, to enhance germplasm for resistance to WYR using APR, to identify new gene/s for rust resistance, to undertake Genome-wide Association Studies (GWAS) of resistance to WYR across partner country environments, to enable pathogen surveillance for minor gene virulence by developing near isogenic wheat stocks, and to build partner capacity in rust pathology and genetics.

More than 3,000 wheat genotypes from diverse locations and synthetics were assembled and screened for stripe rust resistance at PBI Cobbitty. A “Core Set” comprising of 250 wheat genotypes (50 from each partner country) with Adult Plant Resistance(APR) and of reasonable agronomic performance was selected. Additional sets of resistant 300 older wheat genotypes and 94 non-lodging synthetic wheats with unique pedigrees were also selected. Single plant increased seed was tested across environments in partner countries and in greenhouse multi-pathotype tests, and all were genotyped for markers linked to stripe rust APR genes *Yr18*, *Yr36* and *Yr46*. Of the 250 Core Set genotypes, 147 expressed resistance against all three wheat rusts in Australia. Fifty of the Core Set genotypes carried APR,due to the presence of genes *Yr18*, *Yr46*, or uncharacterized APR (UAPR). The Core Set was tested by all partners across multiple years, with a GWAS analysis of the data generated revealing significant QTL on chromosomes 2A, 3A, 5A, 6A, 7A, 1B, 2B, 4B, 5B and 7B. In the Core Set panel, 20 significant QTL were identified in total, 18 of which were present in the subset of 50 genotypes with APR, and 47 unique QTL combinations were detected. A Nested Association Mapping (NAM) population was developed through backcrossing donors of high value stripe rust resistance to susceptible but preferred cultivars selected from each partner country. Our studies identified 127 NAM recombinant lines (RLs) that carried very high levels of APR, and 84 with all stage resistance (ASR). Genotyping of the NAM population was completed and filtration of data and physical alignment with the Chinese Spring genome is in progress. GWAS of the NAM population will allow the identification of markers linked to the high value

genes. Five BC_6F_5 near isogenic lines (NILs) carrying either designated (*Yr36*) or undesignated (*YrCK1*, *YrCK2*, *YrCD*, *YrH*) stripe rust APR genes were generated in the Avocet +*Yr18* background, and different APR genes combinations were produced. All of these APR genes, singly or in combination, provided significantly better protection against WYR as compared to the Avocet +*Yr18* alone. Among the NILs and APR gene combinations, Avocet + *Yr36* and Avocet + *Yr18* +*Yr46* proved to be highly resistant against WYR. Although all 94 synthetics and 300 selected wheat genotypes were resistant in Australia, more than 50% were vulnerable to stripe rust in SA. Twelve doubled haploid (DH) populations were developed that segregated for resistance to WYR in all the environments in which they were tested. Project partners selected rust resistant genotypes from the germplasm.

Junior scientists from the partner countries were trained in Rust Pathology and Genetics, at PBI. Unfortunately, due to circumstances beyond our control, all trainees were male. We did however support several female scientists from the partner countries to attend workshops and conferences. COVID-19 restrictions disrupted travel to the partner countries and although the team was able to progress project activities the capacity to do so was reduced. Advice and germplasm were provided to a private company to promote adoption of project outputs. Newsletters were written and circulated on project activities and a paper was published in a peer reviewed journal. Benefits from the project will continue to flow over the coming years, reducing the impact of WYR epidemics and leading to increased productivity and lowering or eliminating the use of fungicides and resulting in safer food and a cleaner environment.

3 Background

Wheat provides about 20% of world food. It is the most important source of protein and is second only to rice as a source of calories in the developing world. Wheat is an especially important contributor to food security and income for smallholder farmers in Ethiopia, India, Nepal and Pakistan.

Among the pathogens that attack wheat, the rusts are the most feared. They are transboundary pathogens, migrating freely and rapidly on wind currents across thousands of kilometers.

Efficient rust control based on genetics must therefore be regional in scope, ideally targeting epidemiological zones rather than specific countries. It is known that rust pathogens migrate freely within South Asia (SA) and East Africa (EA), and circumstantial but convincing evidence supports periodic migration of rust pathogens between these two epidemiological zones. Stripe rust (WYR) has caused devastating epidemics in wheat crops in SA, including India, Pakistan, Nepal, Bangladesh and Myanmar, and in EA it is a serious problem in the highlands. WYR has been especially problematic in Ethiopia; a severe epidemic in 2010 affected over 400,000 hectares across three regions (Oromya, SNNPR, Amhara). Although the Ethiopian Government distributed fungicides worth about 53 million Birr (AUD $2.5 million) on credit to farmers, severe losses followed and about 500,000 households were affected. The WYR pathogen *Puccinia striiformis* f. sp. *tritici* (*Pst*) was first detected in Australia in 1979 (pathotype (pt. 104 E137 A-), and since then three further incursions of exotic isolates have occurred: one in 2002 (pt. 134 E16 A+); one in 2017 (pt. 239 E237 A-17+ 33+); one in 2018 (pt. 198 E16 A+ J+T+17+) (Ding *et al*., 2021). Each of these incursions has resulted in huge costs to the Australian wheat industry, with up to $90 million being spent on fungicides in some years. Working with the Global Rust Reference Centre at Aarhus University in Denmark, we were able to place each of the four exotic incursions of *Pst* into a DNA-based Multi-locus Genotype (MLG) and determine their likely origins: 1979, MLG PstS0, Europe; 2002, MLG PstS1, North America; 2017, MLG PstS10, Europe; MLG PstS13, Europe or South America (Ding *et al*., 2021).

Beddow *et al.* (2015) estimated that globally, WYR was responsible for annual losses of about 5.47 million tonnes of wheat grain (US$979 million), with a 90% chance of at least 4.70 million tonnes (US$842 million) being lost in any given year. Their estimates showed that millions of tonnes of wheat production is vulnerable to WYR in India, Pakistan, Sub-Saharan Africa and Australasia.

Like many fungal pathogens of broadacre crops, the rusts have been controlled traditionally by integrating cultural practices (*eg* green bridge control) and genetic resistance. The past 30 years has seen increasing use of fungicides in cereal crops, both in the developed and

developing worlds, due to ongoing problems with foliar diseases and patent expiry leading to reductions in chemical prices. The increased use of chemicals in disease control has led to safety concerns surrounding the environment, people handling chemicals, and the potential for chemical residues to enter the food chain. Annual monitoring of Maximum Permissible Residue Limits (MRLs) for fungicides in wheat in Australia during 2013-14 found 9 cases in which the MRLs for the fungicides flutriafol and thiabendazole were exceeded. Reducing or even eliminating the need for fungicides will result in safer food for consumers, a safer workplace, and a cleaner environment. The increased use of fungicides has also resulted in the emergence of isolates of pathogens of plants and humans with insensitivity to the chemicals being used, principally the triazole (Demethylation Inhibitors or DMI, also used in human and veterinary medicine) and Quinone outside Inhibitors (QoI) based chemistries. The ongoing reduction in the spectrum of pathogens that can be controlled by fungicides makes them less appealing. Although insensitivity to fungicides has not been detected in *Pst*, high frequencies of fungicide resistance-associated mutations have been reported from China and New Zealand (Nicola *et al*. 2021). More recently, insensitivity to 4 DMI fungicides was detected in Australia in the leaf rust pathogens of barley and wheat, and in stripe rust, showing that rust fungi can and do acquire insensitivity to fungicides (Park RF *et al*., unpublished).

This multilateral research project was formulated in line with the framework outlined in the ACIAR Strategic Plan 2014-18. It involved partnerships with four countries within the geographic focus of ACIAR, with a research focus on crop improvement. The project delivered outputs that are aligned with priorities articulated in the Strategic Plan, including new tools to increase the speed with which improved wheat cultivars are developed, new knowledge of rust resistance durability in wheat and of rust pathogen variability, and enhanced research capacity in all partner countries. Ultimately, this will contribute to more sustainable and productive agriculture, benefitting all partner and Australian stakeholders especially via increased preparedness for potential future incursions of *Pst*.

The ultimate goal of the project was to generate knowledge, improved germplasm and new tools to enable wheat breeding programs to develop higher yielding, disease resistant wheat cultivars, and new diagnostic pathogen tools to assist with post-management release and pre-emptive resistance breeding. Clearly, to be effective in increasing and stabilising wheat production, the project outputs need to be integrated into existing rust control and wheat improvement efforts in each partner country. The intention of this project was not to produce germplasm that could be directly released to farmers, but rather improved germplasm for use in local breeding programs. Within project training of both breeders and pathologists was targeted to encourage engagement and ownership of the germplasm and tools developed so as to optimise uptake by breeding programs of not only germplasm, but also the tools

developed. Furthermore, the knowledge imparted in training will mean that staff are better equipped in knowing how to apply project outputs in rust control.

Previous ACIAR projects that included rust control in wheat are:

CSI/1983/037 and CSI/1988/014: on genetics and breeding for rust resistance in wheat targeted leaf rust and stem rust in India and Pakistan. This project included genetic analyses of resistance to these two rust pathogens in wheat, and also undertook capacity building. An Impact Assessment on the project undertaken in 2004 estimated that this project had a benefit: cost ratio of 17.3, with benefits estimated at AUD$57.2 million (Brennan JP, Quade KJ (2004). Genetics of and breeding for rust resistance in wheat in India and Pakistan. ACIAR Impact Assessment Series Report No. 25). A major legacy of this project was a book on wheat rust diseases and breeding for resistance, “McIntosh RA, Wellings CR, Park RF (1995). Wheat Rusts: an Atlas of Resistance Genes”, 200pp. CSIRO Publishing: Melbourne, the cost of production of which was underwritten by the projects. This book had a print run of 1,500 and sold out. It continues to be cited very widely (over 1,900 citations to date) and was scanned (with permission) and made freely available for download, using funds provided by the Bill and Melinda Gates Foundation (http://www.globalrust.org/traction). This book is now 27 years old and although its content remains relevant it requires updating.

CIM-2003-067: ‘Ensuring productivity and food security through sustainable control of wheat yellow rust in Asia’ ended 31/12/2009. This project targeted stripe rust of wheat in 12 Asian countries (China, India, Pakistan, Nepal, Afghanistan, Iran, Kazakhstan, Uzbekistan, Azerbaijan, Kyrgyzstan, Tajikistan, and Iraq). It extended a near isogenic series of major stripe rust resistance genes and used them in field (trap) nurseries to monitor virulence for each gene. It also facilitated the development and trialing of high yielding adapted wheat germplasm believed to carry durable minor gene resistance to stripe rust, principally for China but also Afghanistan, Egypt and Ethiopia. Scientists at various stages of their careers were trained in rust pathology and genetics either by in-country workshops (Uzbekistan and Tunisia) or at CIMMYT/ PBI (12 scientists from China, India, Iran, Kazakhstan, and Pakistan). Since this project ended, new technologies such as GBS-based platforms for pathogen diagnostics and host resistance characterisation have become available. New challenges have also emerged, including the global spread of new aggressive pathotypes of *Pst* that have in some cases eroded the effectiveness of the minor gene resistance targeted in this previous project. In addition, several of the scientists trained in the project have retired or moved to new positions, highlighting the need for further training of the next generation scientists in these countries. The final report from this project recommended that work on “pathology, genetics, breeding, training and seed dissemination need to continue to ensure new research results reach farmers to enhance their income and contribute to food security”.

The outputs of this project provided a sound foundation on which to apply new technologies and to extend reach and impact into EA and SA.

CIM-2013-009 (an extension of CIM-2005-020): molecular marker technologies for faster wheat breeding in India – Phase 2 ended 30/06/2017. The principal aim of this project was the implementation of Marker Assisted Selection (MAS) in Indian wheat breeding programs. The traits being targeted for MAS included rust resistance. While the current project included India, its geographic focus was much broader, with the role of India being to provide backstopping for SA. Unlike CIM-2013-009, we did not generate germplasm adapted specifically to India.

Existing relationships and linkages with other organisations were especially helpful in establishing the current project. It benefitted from research being undertaken that the University of Sydney under the GRDC-funded Australian Cereal Rust Control Program (ACRCP), and we also collaborated with Dr Manisha Shankar (Department of Primary Industries and Regional Development, WA), Dr Mandeep Randhawa (CIMMYT, Kenya), Dr Sridhar Bhavani (CIMMYT, Mexico).

4 Objectives

The underlying research theme of this project was to undertake a critical appraisal of the effectiveness of known and unknown minor gene resistance to *Pst* across the diverse environments of EA and SA. Research on WYR genetics over the past 50 years or so has indicated that pyramiding multiple effective resistance genes, maintaining diversity in the resistance genes being used, and responsible gene stewardship, is the optimal strategy to achieve sustained control to this disease. This research also revealed the greater durability of minor gene (Adult Plant) resistance (APR) compared to major gene (All Stage) resistance (ASR). APR to WYR is being increasingly relied upon in many countries including Australia, however, in some cases it has not provided levels of protection adequate enough to prevent yield loss. This could be due to which and how many APR genes are present in a variety, environmental factors (*eg* temperature, pathogen inoculum load), and/or pathogen virulence for the APR. This project is the first time anyone has examined the contribution of these factors to the level of protection provided by minor gene APR. To do this, it focused on determining how diverse the genetic basis of resistance to *Pst* is in wheat in our partner countries, identifying sources of APR that were stable across environments and over years for use in developing adapted wheat germplasm with improved durable resistance to stripe rust, and what the genetic basis of those resistances. Efforts to understand variability in pathogen virulence for minor gene APR were initiated by developing Near Isogenic stocks (NILs) carrying one or more known or partially characterised APR genes for use in pathogen monitoring. These stocks will also serve as the building blocks to develop 3+ gene combination NILs, which can be assessed under different environmental conditions to precisely determine the protective value of minor APR genes singly and in different combinations.

We anticipated significant spill-over benefits to Australia by increased preparedness for potential future incursions of WYR, by identifying markers linked to effective resistance genes that can be used in pre-emptive breeding and via the development of genetic stocks that allow rapid assessment of the effectiveness of important resistances to exotic rust incursions. The specific project objectives were to:

Characterise resistance to WYR in selected germplasm ("Core Set") from partner countries:

- Greenhouse multi-pathotype testing in Australia using an array of *Pst* pathotypes to characterise the ASR genes present.
- Postulation of APR genes based on marker genotyping.
- Field testing across environments in Australia, EA and SA and GWAS

- Selection of the most promising genotypes from Core Set for use in local breeding programs.
- Development of mapping populations based on high value APR, rust screening across environments and genotyping to map the resistances present.

Validate minor gene combinations and refine markers:

- Generation of a Nested Associated Mapping (NAM) population based on elite minor gene donors and reference cultivars selected to represent EA and SA.
- Genotyping-by-Sequencing (GBS) of the NAM population and phenotyping in all partner countries.
- GWAS of the NAM population and identification of linked markers.
- Selection of the most promising NAM lines by each partner for use in local breeding programs.

Develop NIL stocks carrying minor APR genes to assist in *Pst* surveillance:

- Provide training in rust surveillance (wheat and barberry), including sample collection and processing.
- In-country race analysis including preservation of important *Pst* isolates in each partner country for use in future pre-breeding activities.
- SSR genetic fingerprinting (at PBI) of isolates of *Pst* collected annually in participating countries.
- Development of new diagnostic tools for *Pst* based on either DArT-seq technology or SNP chip technology to allow assessments of the degree of genetic diversity and mechanisms driving pathogen evolution.
- Development of a set of NILs to complement the current Avocet series, to allow precise assessments of pathogen virulence for minor resistance genes both in the field and greenhouse.

Train key personnel from partner countries to build partner capacity in rust pathology and genetics:

- To foster the network of trained personnel in the partner countries, five months training of up to 8 young junior scientists (pathologists and breeders) at PBI. First cohort of four junior scientists completing training during year 1 of the project. Second group of four junior scientists completing training during 2nd year of the project.

5 Methodology

Wheat germplasm

Altogether, 3,000 geographically diverse wheat genotypes including 766 synthetic hexaploid lines were sourced from germplasm collections at the Australian Grains Genebank and the Plant Breeding Institute (PBI). A “Core Set” containing 250 wheat cultivars (50 from each partner country), an additional set of 300 old wheat genotypes and cultivars and 94 synthetics were selected from the 3,000 genotypes based on reasonable agronomic type and an initial disease screen that established the presence of APR to *Pst* in each. From the remaining 2,750 genotypes, we selected a further 624 genotypes (2017) and 395 (2018), from which 300 of the best performing genotypes were chosen. All the 766 hexaploid synthetic wheat genotypes including primary crosses of *Triticum turgidum* x *Ae*. *tauschii* Coss. (= *Ae*. *squarrosa* L.) and their derivatives, which were initially developed at CIMMYT (Mujeeb-Kazi *et al*. 1996) were assessed for rust resistance and agronomic characters. Along with other wheat genotypes, synthetics were screened for rust resistance in artificially inoculated rust field nurseries for three seasons consecutively. A total of 591 synthetic genotypes were consistently resistant to WYR, from which a subset of 94 non-lodging genotypes with unique pedigrees was established. Details of the Core Set of 250 genotypes, an additional set of 300 old wheat genotypes, and the subset of 94 non-lodging synthetic wheat genotypes are provided in **Appendices I**, **II** and **III**, respectively. Control genotypes including susceptible (Avocet S) and a *Yr18* NIL in Avocet S background were sourced from seed collections maintained at the PBI.

Under greenhouse conditions at PBI, the “Core Set” and 94 synthetics were further increased by selecting randomly a single plant from each line. Seed derived from these single plants was used for testing across the environments, greenhouse multi-pathotype testing and for DNA extractions for genotyping using markers linked with APR genes *Yr18*, *Yr36* and *Yr46*.

Fig. 2: Single plant seed increase in the greenhouse at PBI

Pathogen material

For greenhouse seedling multi-pathotype testing to detect and identify ASR genes, seven or more Australian pathotypes of *Pst* (104 E137 A+, 110 E143 A+, 134 E16 A+, 134 E16 A+ 17+

27+, 134 E16 A+ J+ Yr27+, 134 E16 A+ J+ T+, 150 E16 A+, 239 E237 A- 17+ 33+ and 198 E16 A+ J+ T+ 17+) were used. A mixture of pathotypes selected to represent those prevailing in Australian grain growing regions was used to inoculate field trials of the germplasm. The virulence patterns of the Australian pts against seedling resistance (*Yr*) genes are detailed in **Appendix IV**. Apart from field trials at Karnal (India), which were artificially inoculated using pathotypes prevailing in that area, those in Ethiopia, India, Nepal and Pakistan were exposed to natural inoculum.

Field rust screening

The Core Set, additional wheat genotypes and the synthetics were phenotyped for *Pst* resistance across all partner countries during the 2018, 2019 and 2020 cropping seasons, and additional data was collected from PBI Cobbitty trials during 2017 and 2021. The Core Set was also tested for two years in Western Australia (WA).

All genotypes were sown as one-meter rows during June (Australia), May (Ethiopia and Kenya) and in November to early January (India, Nepal and Pakistan). A row of susceptible spreader (mixture of stripe rust-susceptible wheat genotypes) was sown after every fifth test line row to allow the build-up and uniform distribution of *Pst* inoculum. Four weeks after sowing, plots were fertilised using granular urea (w/w 46% nitrogen @ 100 kg/hectare) followed by irrigation. In Australia, plots were irrigated twice a week or as required, using fixed sprinklers. In other countries, plots were irrigated using flood irrigation.

In Australia, field epidemics of stripe rust were created following the procedures described by McIntosh *et al*. (1995). Urediniospores (30–40 mg) were suspended in 1.5 L of light mineral oil (Shellsol®, Mobil Oil) and sprayed over buffer/spreader genotypes with an ultra-low-volume applicator (Microfit®, Micron Sprayer Ltd., UK). Four to five inoculations were performed during late evening on days that had a strong forecast of overnight dew. On the first and second inoculations, hot spots of disease were established by placing susceptible Morocco plants which were sown in 9 cm diameter pots and already infected in the greenhouse. Research trial area was irrigated at least twice a week using fixed sprinklers, to boost crop growth and to create congenial conditions for stripe rust development. In all other countries, epidemics of stripe rust were dependent on the natural inoculum of pathogen. Adult plant stripe rust response was scored at the flag leaf growth stage in all the crop seasons using 1–9 scale developed by Sandhu *et al*. (2021), as explained in **Fig. 2** and **Table 1**.

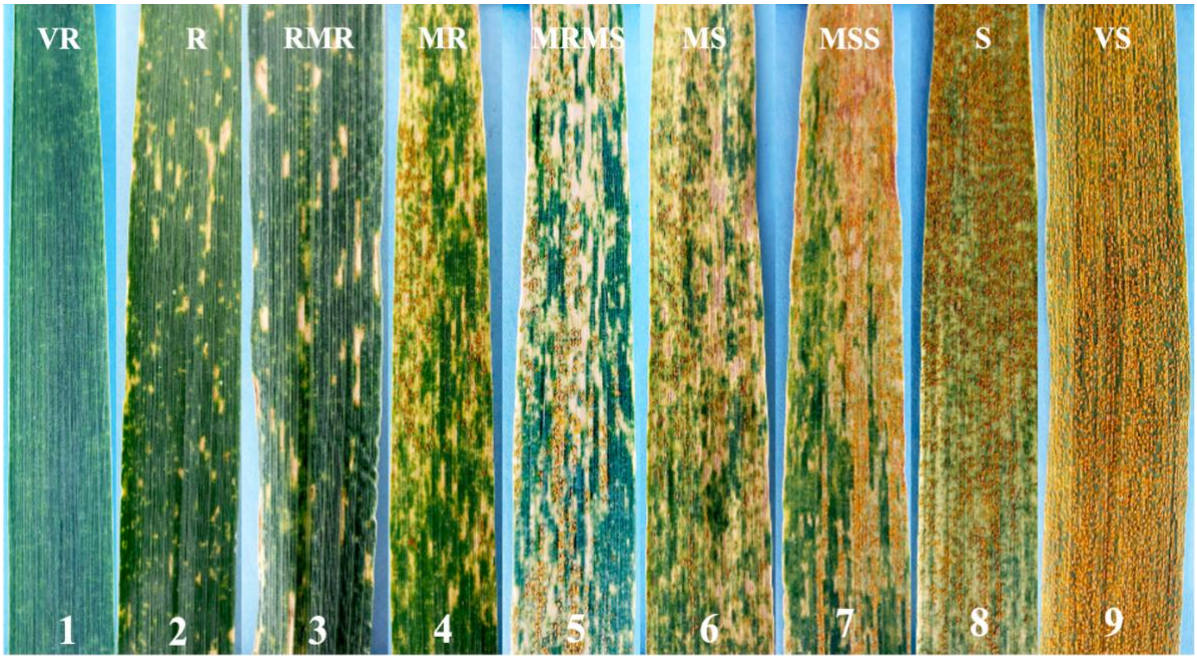

Fig. 2: Field rust scale (1–9) (Sandhu *et al*. 2021)

Table 1: Description of the 1 to 9 field scale used for scoring wheat yellow rust response

Scale	Description	Host response on flag leaf
1	VR (Very Resistant)	No visible infection or chlorosis/necrosis, 100% leaf area green, minute flecks may be present
2	R (Resistant)	No visible uredinia, minute chlorotic/necrotic flecks present, > 90% leaf area green
3	R–MR (Resistant to Moderately Resistant)	Chlorotic/necrotic flecks/patches and minute or trace of restricted uredinia with necrosis, > 80% leaf area green
4	MR (Moderately Resistant)	Small uredinia with slight sporulation with chlorosis and necrosis present, > 70% leaf area green
5	MR–MS (Moderately Resistant to Moderately Susceptible)	Small to medium uredinia with moderate sporulation and chlorosis/necrosis present, > 60% leaf area green
6	MS (Moderately Susceptible)	Medium uredinia with moderate sporulation and some chlorosis/necrosis present, approximately 40% leaf area green
7	MS–S (Moderately Susceptible to Susceptible)	Medium uredinia covering up to 70% of leaf area with moderate to high sporulation with little chlorosis/necrosis
8	S (Susceptible)	Large uredinia covering up to 80% of leaf area with abundant sporulation and no chlorosis/necrosis
9	VS (Very susceptible)	Very large and severe uredinia covering >90% of leaf area with extreme sporulation and with no chlorosis/necrosis

Source: Sandhu *et al*. (2021)

A total of 33 data sets were recorded for the Core Set across environments in Australia (Cobbitty NSW, Manjimup WA), Ethiopia (Bekoji, Debre Zeit, Kulumsa, Meraro), India (Jammu, Karnal), Nepal (Kabre, Khumaltar), Pakistan (Attock, Faislabad, Islamabad, Narrowal, Nowshera) and Kenya (CIMMYT, Njoro).

Fig. 3: Field rust screening at (**A**) Horse Unit Cobbitty, NSW Australia (**B**) Crop Disease Research Institute, Islamabad, Pakistan (**C**) Kulumsa Agricultural Research Centre, Ethiopia **(D)** CIMMYT rust screening facilities, Njoro, Kenya

Greenhouse multi-pathotype testing

All greenhouse rust screening was conducted at PBI in Australia. For greenhouse tests, all genotypes along with differentials (Appendix V) were planted in pots filled with a mixture of fine bark and coarse sand and fertilised using “Aquasol®” (100 gm per 10 litres of water per 200 pots) prior to sowing. Seedlings of differentials and test genotypes/genotypes were raised in 9 cm diameter pots by sowing four clumps (test genotypes) of each genotype using 8–10 seeds per clump. Following sowing, pots were kept in a growth room at 20 ± 2°C for germination. Seven-day old seedlings were fertilised with granular urea using “Incitec Pivot” w/w 46% nitrogen (50 gm per 10 litres of water per 200 pots). Seedlings at the one and a half to two leaf growth stage (10–12 days old) were inoculated with a suspension of *Pst* urediniospores (2 mg urediniospores/1.0 ml of light mineral oil; Univar Solvent Naphtha L 100), using an airbrush

attached to a motorized compressor. Upon atomisation of rust suspension, door of the inoculation room was kept closed for 5 min to allow urediniospores to settle on the leaves. Plants were then transferred to incubation cabinets fitted with misters which maintained greater than 95% relative humidity in a dark room where they were incubated. Incubation was carried out at 10^{o}C for 24 h in the dark room installed with cabinets. Post incubation, plants were moved to naturally lit microclimate rooms maintained at 18 ± 2^{o}C. Greenhouse Infection type responses were scored 12–14 days after inoculation according to the "0"–"4" scale used by Park and Karakousis (2002), described in **Appendix VI**.

Phenotyping with additional pathogens

Germplasm including the Core Set, the NAM population, additional wheat germplasm and the synthetics were also phenotyped for resistance against leaf rust (WLR) and stem rust (WSR) at PBI. Methods used for evaluations against these two diseases were as described for WYR. The Core Set was also evaluated for WLR resistance at Debre Zeit (Ethiopia) and Indore (India). Also, multi-pathotype testing of the Core Set for ASR to WLR resistance was completed at PBI, providing the data needed for GWAS analyses of resistance to this disease. The Core Set was also tested for yellow leaf spot (YLS; aka tan spot (*Pyrenophora tritici-repentis*) resistance under field conditions in Western Australia. Screening of the Core Set for wheat blast resistance is currently under progress in Bangladesh, being coordinated by CIMMYT.

Genotyping using markers linked to known APR genes

DNA was extracted from the same single plant, from which seed was increased for each line. Genotyping was conducted at PBI, using markers linked with stripe rust APR genes *Yr18*, *Yr36* and *Yr46*. Two of these APR genes also confer APR to stem rust, leaf rust and stripe rust with each locus being designated *Yr18*/*Lr34*/*Sr57*/*Pm38* and *Yr46*/*Lr67*/*Sr55*/*Pm46*.

DNA Extraction

Genomic DNA was extracted from leaf tissue using a CTAB (cetyltrimethylammonium bromide) protocol (Doyle and Doyle 1990). A 15 - 20 mm sample of leaf tissue was collected from 10-12-day old seedlings into 2 ml Eppendorf tubes from each test line and controls. The tubes were kept for 96 hours above silica beads to dry the leaf tissue. Silica beads were replaced twice with the dried ones during the drying of leaf tissue samples. Two small stainless steel ball bearings were added per tube and dried leaves were crushed to powder using a Retsch MM300 Mixer Mill (Retsch, Germany) for 3 min at 25 rpm. Pre warmed (65°C) 700 µl of CTAB extraction buffer was added per tube. Samples were incubated for 30 min at 65°C and tubes were shaken vigorously after every 10 min of incubation. An equal amount of

Chloroform: phenol (700 µl, 24:1 v/v) was added per tube and the contents were mixed properly by inverting each tube at least 100 times. Samples were centrifuged for 15 min at 12,000 rpm and 650 µl of supernatant was transferred to new 1.5 ml tubes. An equal volume (750 µl) of chilled (-20°C) isopropanol was added per tube and the supernatant was mixed thoroughly by inverting tubes several times. The tubes were placed in a freezer (-20°C) for 10 min to precipitate DNA and then centrifuged at 10,000 rpm for 10 min to pellet the DNA. The supernatant was discarded, and the pellet was washed with 500 µl of of wash buffer (76 % v/v ethanol and 10 mM ammonium acetate). DNA pellet was air dried and re-suspended in 200 µl of 10 mM Tris-HCl (pH 8.0). Rnase A @ 20 µl per 40 ml of 10 mM Tris-HCl was added before the re-suspension of DNA pellet. Tubes were kept in an oven (37°C) for 2 hrs to dissolve the DNA pellet properly. DNA was quantified using a Nanodrop ND-1000 spectrophotometer (Nanodrop® Technologies). Working dilutions (30 ng/µl) of all samples were prepared from these stocks, using doubled distilled autoclaved water (ddH_2O).

Genotyping using molecular markers

The Core Set, synthetics and control genotypes were genotyped with three gel-based markers and one Kompetitive allele specific PCR (KASP) marker linked with APR genes. The codominant microsatellite marker csLV34 (Lagudah *et al*. 2006) linked with *Yr18*, gene-specific primers WKS1 and WKS2 (Fu *et al*. 2009; Huang *et al*. 2016) linked with *Yr36* and KASP marker TM4 linked with *Yr46* (Moore *et al*. 2015) were used in genotyping. Details of all markers and PCR profiles used in this study are provided in **Appendix VII**.

For gel-based markers, PCR was performed using 10 µl of reaction volume containing 3.0 µl of genomic DNA (30 ng μl^{-1}), 2.0 µl of 5x MyFi™ (Bioline Australia Pty. Ltd. Alexandria NSW) reaction buffer formulation (containing dNTPs and $MgCl_2$), 0.25 µl of each forward and reverse primer, 0.1 µl (5 U μl^{-1}) of *Taq* (MyFi™ DNA Polymerase, Bioline Australia Pty. Ltd. Alexandria NSW) and 4.4 µl of ddH_2O. Reactions were performed in a 96-well DNA thermocycler (Eppendorf AG 22331 Mastercycler, Hamburg, Germany). PCR products were resolved on 2% (w v-1) agarose (Agarose, Molecular Grade, Bioline Australia Pty. Ltd. Alexandria NSW) gels at 110 V electrophoresis for 1.5 hrs. For staining, 2.0 µl of GelRed™ (Biotium Inc., CA, USA) was added per 100 ml of gel solution. One hundred bp HyperLadder™ IV (Bioline Australia Pty. Ltd. Alexandria NSW) was used as molecular size marker. The separated fragments were visualized under an ultraviolet light unit fitted with a GelDoc-IT UVP camera.

KASP assay was performed using 8.0 µl of reaction volume containing 3.0 µl of genomic DNA (30 ng μl^{-1}), 4.0 µl of 2 × KASP-TF Master Mix [(optimised buffer containing universal fluorescence resonance energy transfer assettes for FAM and HEX, ROX™ passive reference dye, Taq polymerase, nucleotides and $MgCl_2$), (LGC Biosearch Technologies)],

0.11 µl primer mix (mixture of 12 µM each allele-specific A1 and A2 primers and 30 µM of common reverse primer) and 0.89 µl of autoclaved ddH2O. PCR reactions were performed in T100™ thermal cycler (BioRad, USA) using a 96-well PCR plate. Amplification conditions included 15 min at 94 °C; 10 touchdown (TD) cycles of 20 s at 94 °C, 60 s at 65–57 °C (dropping annealing temperature by 0.8 °C per cycle); and 35–38 cycles of 20 s at 94 °C, 60 s at 57 °C. End product fluorescence readings were performed at 25 °C for 30 s using a CFX96 Touch™ real-time PCR detection system (BioRad, USA).

Genotyping of Core Set entries using 90KSNP chip

With the aim to identify QTLs for resistance to WYR from a diverse Core Set of genotypes originating from Australia, Ethiopia, India, Nepal and Pakistan, genotyping was accomplished using the 90K SNP iSelect bead chip for wheat. DNA (50 ng μl^{-1}) from each of the 250 Core Set genotypes was sent to Agriculture Victoria Research, Department of Jobs, Precincts and Regions, Victoria, Australia. Using GWAS, QTLs associated with resistance to WYR were identified from imputed marker data in conjunction with the field data collected from evaluations across the environments.

Development and genotyping of a NAM population

A NAM population was developed to provide improved germplasm to all partners, and to enable the development of markers linked to high value WYR APR genes to facilitate their use in pre-emptive breeding. Popular wheat cultivars in each partner country with vulnerability to WYR were selected and used as recurrent parents: Enkoy, Digelu, Danphe and Picaflor (Ethiopia); DBW 17, HD 2733 and PBW 343 (India); Gautam and NL 971 (Nepal); Inqilab 91 and Sehar 2006 (Pakistan). Resistant donors including Baxter, Diamondbird, Kingbird, Sunlin, Synthetic AUS30515 and 7SRRSN Line ISR1043.15 were selected based on robust APR to WYR in field tests in Australia. Initially, 61 crosses were made between the recurrent parents and the donors. F_1s from Sunlin crosses turned out to be grass clumps (associated with grass clump complementary genes) and were hence discarded. The F_1s derived from 40 crosses were each backcrossed with the recurrent parents to produce BC_1F_1 (**Fig. 4A**).

Individual BC_1F_1 plants were harvested and equal numbers of F_2 seeds were pooled from each cross. Up to 200 seeds from each F_2 family were space planted as long rows at the PBI research field site. Plants with good agronomic characters were selected by culling tall, lodged, and bushy genotypes. From each long row, selected plants were harvested individually. BC_1F_3 genotypes were raised by accelerated generation in controlled environment rooms at PBI over the 2018/19 summer (**Fig. 4B**). In the 2019 cropping season,

more than 2,200 BC_1F_4 families were sown in the field and BC_1F_5 seed of each recombinant was harvested. The plan adopted for the development of NAM population is described in **Fig. 5**. Further, population size was reduced to keep the genotyping cost within the budget available. By the end of 2019, the NAM population comprised a selection of 1,452 BC_1F_5 RLs developed from different 28 crosses, which were distributed among the partner countries. The NAM population was screened for rust resistance under field conditions in Australia, Ethiopia, India, Nepal and Pakistan for 2 years, enabling partners to select the most promising genotypes for use in local wheat breeding programs.

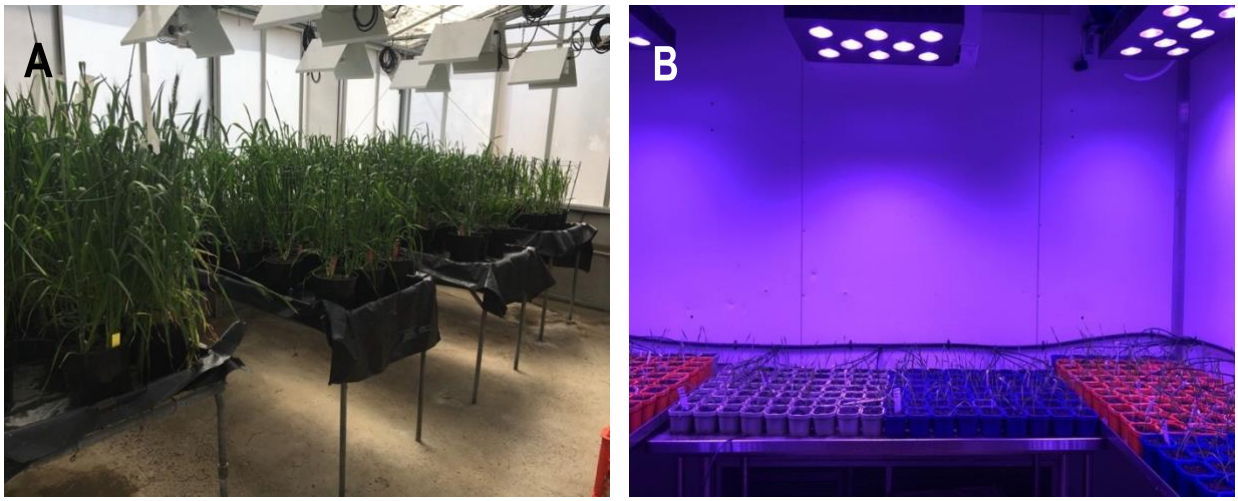

Fig. 4: Development of NAM population (**A**) BC_1F_1s under greenhouse conditions at PBI (**B**) BC_1F_3 genotypes growing in the controlled environment room at PBI.

Randomly, single BC_1F_5 seed of each of the 1452 genotypes and parents was selected for DNA extractions. Sequencing of the NAM population was completed by the Geneshifters USA using Illumina Platform with PE150 bp read lengths with minimum coverage of 200K reads/sample. Filtration of sequencing data and physical alignment with Chinese Spring genome is under progress. GWAS of the NAM population will allow the identification of markers linked to the stripe rust APR genes that provide adequate levels of resistance across different environments to facilitate their use in resistance breeding.

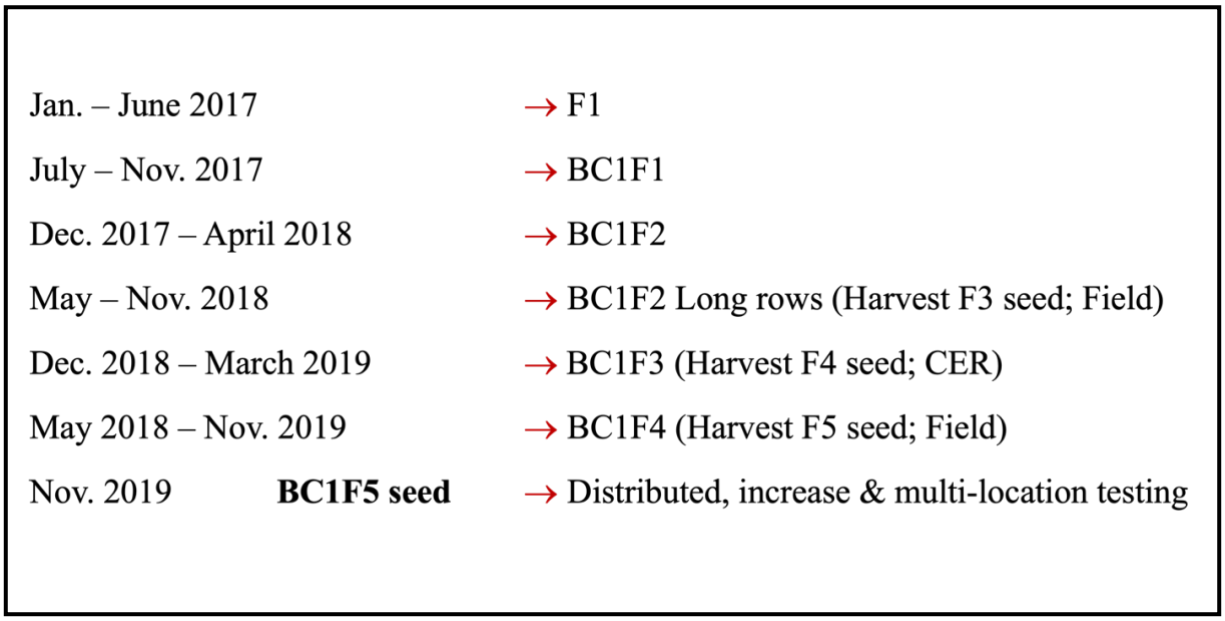

Fig. 5: Timeline for the development and distribution of the NAM population

Development of NIL stocks

Near isogenic genotypes (NILs) carrying stripe rust APR genes (*YrCK1*, *YrCK2*, *YrCD*, *Yr36* and *YrH*) in the Avocet + *Yr18* background were developed through backcrossing. Donors of four of these five APR genes were selected from existing Doubled Haploid (DH) populations developed at PBI: *YrCK1* (Cook x Avocet S DH Line: CA61); *YrCK2* (Cook x Avocet S DH Line CA51); *YrCD* (Capelle Desprez x Avocet S DH Line: CD233); *YrH* (Hereward x Avocet S DH Line: HA 28). The hard red spring wheat cultivar Lillian from Canada was used as the donor for *Yr36*. The line carrying each APR gene was crossed with the NIL stock Avocet + *Yr18*. Stripe rust resistant F_1s were selected based on rust testing at the adult plant growth stage and genotyping using *Yr18* linked marker csLV34 (Lagudah *et al*. 2006; **Fig. 6**). F_1s carrying *Yr18* linked marker were rust tested, and the most resistant plant back crossed to Avocet + *Yr18.* The backcrossing continued until the BC_6F_1s generation for each APR gene. BC_6F_1 families were allowed to self and offspring were selected through rust testing at the adult plant growth stage and genotyping with *Yr18* linked marker. Finally, seed of each BC_6F_5 NIL carrying APR was distributed. In addition, NILs carrying combinations of APR genes *Yr18 +Yr29*, *Yr18 +Yr46*, *Yr18+Yr29+Yr46* and *Yr29 +Yr46* were produced and distributed. All of the NILs were tested at adult plant growth stages under field conditions and in the greenhouse at PBI. All the APR genes (*YrCK1*, *YrCK2*, *YrCD*, *Yr36* and *YrH*) and combinations provided significantly better protection against WYR than did *Yr18* alone.

Although it was not a part of the project, we undertook the development of an additional 22 NILs carrying known APR genes for stripe rust resistance. These NILs require further advancement and testing.

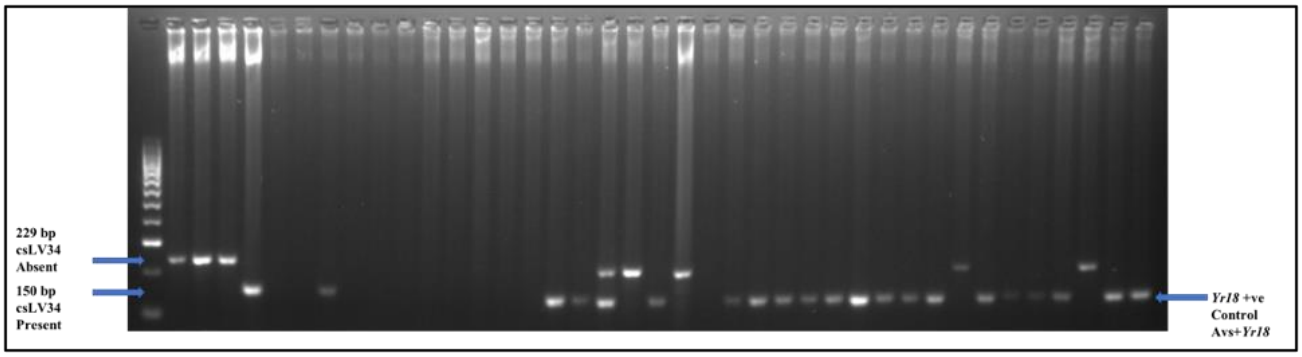

Fig. 6: Genotyping using *Yr18* linked marker csLV34 during the backcross process

These NIL stocks will allow precise assessments of pathogen virulence for minor resistance genes, both in the greenhouse and under field conditions. These NILs and combinations of APR genes can reveal the level of protection provided by specific stripe rust resistance genes singly and in combination under field conditions.

Development of mapping populations

To map new stripe rust resistance genes in wheat, 12 doubled haploid (DH) populations were developed at PBI (**Fig. 7**). Twelve parents carrying unidentified APR or ASR to stripe rust were selected from exhaustive rust tests of the Core Set and crossed with susceptible Avocet S.

Details of all parents are provided in **Appendix VIII**. Around 25 to 30 F_1 seeds from each cross were sown. Each F_1 plant was crossed with maize pollen to get more than 400 embryos per cross (Resistant x Susceptible). Heads from the F_1 plants crossed with maize were collected to rescue embryos, which were then grown in tissue culture. Attaining around 50% success rate, more than 200 haploid seedlings per cross were achieved. Haploid seedlings at two to three leaf stage were then treated with colchicine, and the treated plants grown in the greenhouse to develop DH plants. More than 150 DH plants were generated per cross. More than 2,000 DH lines were produced in total, and plants with abnormal growth were rejected. Each DH plant was allowed to self and one to 50 seeds per DH plant were harvested. Further seed of each DH line was increased in the Germplasm Enhancement Facility ((GEF) at PBI. It took almost 2 years to develop these 12 DH populations, details of each are provided in **Appendix IX**. A total 1,788 DH genotypes plus parents with adequate seed were sent to the partners countries except Ethiopia, for which we did not receive a valid

import permit. All DH populations were screened for adult plant field response to WYR at PBI during the crop season 2021 and during 2021-22 in India (selected populations), Nepal and Pakistan. DH populations segregated for WYR resistance when tested under field conditions and further testing is required to map the gene/s for stripe rust resistance carried by the resistant parents.

Fig. 7: Steps involved in the development of doubled haploid populations at PBI

In addition to the field screening for WYR resistance, DH populations were tested for WLR as well at PBI.

Training: Build partners capacity in rust pathology and genetics

To build partner capacity in rust pathology and genetics, we planned to train two cohorts of scientists for five months each at PBI, starting in February and in July 2018. Although the process to identify suitable candidates and make the necessary arrangements for them to be able to visit PBI was started in August 2017, it was not finalised in time due to a series of unavoidable circumstances at the partner countries' end. Accordingly, we decided to train all eight candidates together in one group at PBI, in the beginning of 2019. Six trainees (Mr Dawit Asnake Tigabu and Mr Tamene Mideksa Sarbesa from EIAR, Ethiopia, Dr Satish Kumar and Dr Pramod Prasad from Indian Institute of Wheat & Barley Research (IIWBR), Karnal, India and Mr. Prem Bahadur Magar and Mr Shiwarttan Kumar Gupta from Nepal Agricultural Research Council (NARC), Nepal) attended a five-month training

program at PBI. An additional trainee, Ms Aline Casassola (a student of Bachelor of Agronomy at the University of Passo Fundo, Brazil), was invited to join this training. Unfortunately, two remaining scientists from the Pakistan Agricultural Research Council (Dr Muhammad Fayyaz and Mr Muhammad Sufyan) were unable to attend training due to delays in getting permission at their end. We planned to invite them in 2020, but COVID-19 travel restrictions precluded this.

Scientists from the partner countries were trained in:

- The planning, planting, and rust inoculation of greenhouse rust experiments, and scoring rust infection type responses of greenhouse sown wheat seedlings.
- Techniques for increasing pure isolates of stripe rust and preparation for storage in liquid nitrogen.
- Rust screening of wheat germplasm, using control differential genotypes under greenhouse conditions (**Fig. 8**).
- Evaluation of adult plant stripe rust resistance under field and greenhouse conditions.
- Crossing and speed breeding techniques for the development of wheat populations.
- Preliminary genetic analyses of disease resistance in wheat in greenhouse-based seedling rust testing of segregating wheat populations.
- Identification of rust resistance genes through multi-pathotype testing in the greenhouse.
- DNA extraction and PCR using microsatellite and KASP markers to map rust resistance in research populations phenotyped in the greenhouse.
- Marker assisted selection of adult plant stripe rust resistance genes.
- Cross inoculations and recording infection types and histological examination of host: pathogen interactions.

The training program also included lectures in biosecurity, rust pathology and host: pathogen interactions. Trainees also developed their writing skills by submitting their training outcome reports. At the end, trainees also had the opportunity to visit ACIAR head office in Canberra.

In addition to the training provided at PBI, we also trained partners in the rust screening of wheat germplasm, in the collection and preservation of rust isolates and in conducting cereal rust surveys, during our visits to each of the partner countries (**Fig. 9**). While visiting Nepal, we trained some NARC staff including female scientists and students, in collecting rust samples from different *Berberis* species and preserving them in ethanol.

Identification of genotypes carrying APR and Genome Wide Association Studies

Australian Pts of *Pst* used (**Appendix IV**) in the multi-pathotype testing covered virulence for almost all the known ASR *Yr* genes. Core Set genotypes that were susceptible (infection type “3+”) against Australian pathotypes of *Pst* when tested as seedlings and were resistant ((1 to 7; field scale (Sandhu *et al*. 2021)) at adult plant growth stage in rust screening across the field environments, were considered to carry APR only.

Fig. 8: Trainees learning various techniques to study host: pathogen interactions

Fig. 9: Trainees learning molecular techniques for genotyping of germplasm

Fig. 10: University of Sydney scientists training NARC staff and students in phenotyping under field conditions in Nepal

GWAS was performed on the Core Set using 90Kiselect SNP chip data in conjunction with field data collected from field rust evaluations in Australia, Ethiopia, India, Kenya, Nepal and Pakistan. Association mapping was performed in R (R Core Team 2020) and data were visualised using “ggplot 2” from Wickham (2016). The marker-trait associations for stripe rust resistance were identified from 36,487 SNP markers in conjunction with field rust responses from 33 field evaluations across the environments. Association of loci were considered significant at p-value < 0.001 and the associated loci were visualised through Manhattan Plots using “ggplot 2” (Wickham 2016). Where at least three markers within 20Mbp were found to be significantly associated with resistance, it was considered as a QTL. QTL were named according to the environment in which they were detected (**Table 2**). QTL assignment was considered more confident when higher numbers of markers were associated with resistance. The position of each of the 20 QTL detected was aligned with previously reported *Yr* genes and projected on the consensus maps available at GrainGenes (https://wheat.pw.usda.gov/GG3/ or http://www.graingenes.org).

Data analysis

All the field and greenhouse rust scoring data were recorded using MS Word Excel.

Goodness-of-fit of observed segregation ratios with the expected genetic ratios of phenotypic data from DH populations was tested using Chi-squared ($\chi 2$) analysis.

6 Achievements against activities and outputs/milestones

The main aim of this project was to reduce vulnerability to WYR in EA and SA by establishing, equipping, and mobilising a collaborative network of key cereal improvement centres and a knowledge base in SA and EA that will enable ongoing research and development. The achievements of each objective against project activities and outputs/milestones are reported below:

Objective 1: Characterise resistance to WYR in germplasm from partner countries

No.	Activity	Outputs/ milestones	Completion date	Comments
1.1	Germplasm assembly and field rust screening across the environment to characterise new sources for rust resistance	Germplasm enhancement for rust resistance	Dec. 2020 (All) Dec. 2021 (Additional data from Australia)	A set of 3,000 diverse wheat genotypes was rust tested at PBI in 2017. This field rust screening revealed that 10%, 25%, 35%, 9% and 21% of the genotypes were highly resistant, resistant, moderately resistant, moderately susceptible, and susceptible to WYR, respectively. A Core Set comprising 250 genotypes (50 from each partner country) was selected and tested for five years at PBI and for four years in EA and SA. A subset of 300 genotypes with promising resistance was (also selected from the testing of 3,000 genotypes) tested again under field conditions during the 2018, 2019 & 2020 crop seasons in Australia, EA and SA. From the testing of 766 synthetic wheat genotypes, an additional set of 94 synthetic wheat genotypes was selected and characterized for WYR resistance, across the environments of EA and SA for three years and for five years in Australia.
1.2	Germplasm screening under greenhouse conditions, using an array of *Pst* pathotypes	Germplasm enhancement for rust resistance	Dec. 2020	Core Set genotypes (250), 300 genotypes from subset and 94 synthetic wheat genotypes were tested using a range of Australian pathotypes of *Pst* (**Appendix IV**), under greenhouse conditions at PBI.
1.3	Comparison of greenhouse multi-pathotype testing data and field rust screening data	Identifying new sources of stripe rust adult plant resistance.	Dec. 2020	Genotypes from Core Set (250), subset (300) and synthetics (94), susceptible to all the Australian pathotypes of *Pst* (**Appendix IV**) used in the study that were resistant under field conditions were considered to carry APR only.
1.4	Genotyping of germplasm	Genotyping or the presence of stripe rust resistance APR genes linked markers	Dec. 2020	Core Set (250), subset genotypes (300) and synthetics (94), were genotyped using stripe rust APR linked markers (**Appendix VII**).

1.5	90K SNPChip genotyping of 250 Core Set genotypes	Genotyping to perform GWAS	Dec. 2020	Genotyping of the 250 Core Set genotypes, using 90Kiselect SNP chip was completed. GWAS conducted using multi-location field data collected in different seasons and the 90Kiselect SNP chip data revealed the presence of significant QTLs on chromosomes 2A, 3A, 5A, 6A, 7A, 1B, 2B, 4B, 5B and 7B.

PC = partner country, A = Australia

Objective 2: Validation of minor gene combinations and refinement of markers through Genotyping-by-Sequencing (GBS) of NAM and DH populations

No.	Activity	Outputs/ milestones	Completion date	Comments
2.1	NAM population rust screening for seedling resistance	Greenhouse multi-pathotype testing of NAM population.	Feb. 2021	Greenhouse multi-pathotype testing of 1,466 BC_1F_5 NAM genotypes including parents was completed using an array of *Pst* pathotypes (**Appendix IV**).
2.2	Selections for agronomic traits	Selections for pre breeding.	May 2022	All project partners were able to select the most promising genotypes from the NAM population, for use as resistance donors in their local breeding programs.
2.3	Genotyping of NAM population for GWAS	DNA extraction from BC_1F_5 seed for sequencing of NAM genotypes	May 2021	Illumina Platform Sequencing with PE150 bp read lengths, of 1,466 NAM genotypes including parents was completed. GWAS was initiated using the 2020 crop season data, but GeneShifters were unable to complete it due to COVID lockdowns and are still working on this. Final GWAS should be completed in 2023, and will include 2022 data from SA.
2.4	Phenotyping of NAM population for GWAS	Field rust screening of NAM population across the countries	May 2023	Field rust screening to collect a final data set was completed in Australia. 2021 data from Ethiopia is from a reduced number of genotypes, and 2022 data from Nepal and Pakistan is not reliable. So another season data from EA and SA is required for a reliable GWAS. In the current crop season, the NAM population is under testing at PBI. This phenotypic data will be used in conjunction with sequencing data to perform GWAS.
2.5	Development of DH genotypes	Development of DH populations required for the mapping unknown genes for rust resistance.	Dec. 2021	12 DH populations were developed by crossing resistant parents (**Appendix VIII**) with susceptible Avocet S. Seed of DH genotypes was increased at PBI and distributed to partner countries by December 2021.

2.6	Rust screening of DH genotypes	Field rust screening of DH Genotypes	May 2022	All the 12 DH papulations were tested at PBI in 2021, and selected populations in India, Nepal and Pakistan in 2022. We were unable to send DH populations to Ethiopia due to lack of a valid import permit. Partners should be able increase seed and test these populations for response to local populations of *Pst*.
2.7	Genotyping of DH genotypes	Genotyping by sequencing (GBS)	Dec. 2023	GBS on the DH genotypes was planned subject to the extension of the project and availability of funds. Sequencing data would be used in conjunction with the phenotypic data collected from all countries, to map high value stripe rust APR genes and to develop linked markers. Genotyping and phenotypic data could be available by end of 2023.

PC = partner country, A = Australia

Objective 3: To undertake stripe rust pathogen surveillance and development of NILs stocks carrying minor genes

No.	Activity	Outputs/ milestones	Completion date	Comments
3.1	Stripe rust pathogen surveillance	*Pst* surveillance	Oct. 2021 (Ethiopia) And Feb. 2022 (SA)	Partners in EA and SA were able to collect samples of stripe rust. From Nepal samples were sent to Shimla, and local samples from India and Pakistan were analysed in country at Shimla and Muree respectively.
3.2	Marker Assisted Selection (MAS) of BC_6F_{4s} and seed increase	Selection of minor genes using available markers Seed increase to raise next generation	May 2021	Backcrossing and MAS was completed and seed increase from BC_6F_{4s}
3.3	Backcrossing	NILs carrying minor genes for stripe rust resistance	Dec. 2021	Backcrossing for the development of NILs carrying stripe rust APR genes (*YrCK1*, *YrCK2*, *YrCD*, *Yr36* and *YrH*) in the Avocet+*Yr18* background was completed and seed of BC_6F_5 NILs was increased at PBI. By Dec. 2021, BC_6F_5 seed of each NIL was distributed among the partner countries for multi-location field rust testing, except Ethiopia.
3.4	Greenhouse experiment	Expression of APR genes	June 2022	Comparative adult plant greenhouse tests were conducted at PBI to test the APR expression of each NIL when challenged with *Pst* pathotypes.
3.4	Field experiments	Selection of minor genes through rust screening	Dec. 2021 (Australia) and May 2022 (SA)	BC_6F_5 NILs and different APR genes combinations were tested under field conditions in Australia, India, Nepal and Pakistan.

Objective 4: To build partner capacity in rust pathology and genetics

No.	Activity	Outputs/ milestones	Completion date	Comments
4.1	Training in rust genetics and pathology	Training	June 2019 Sep. 2020	A five-month training course in rust pathology and genetics was completed in 2019. Second training program was planned for the remaining two scientists from Pakistan. Both trainees were supposed to start their training in April 2020 at PBI, but they were not allowed to travel due to COVID-19 lockdown.

Objective 5: Project management

No.	Activity	Outputs/ milestones	Completion date	Comments
5.1	Project meeting	Annual meeting	2017, 2018, 2019, 2020, 2021	Face to face annual meetings were conducted, except online Zoom meeting was held on 16th Dec. 2020. Also, the research results were presented at All India wheat and barley workers virtual meet in 2021.
5.2	Project payments	Research expenses	March 2022	All the project payments for research expenses to each partner country have been completed.
5.3	Project payments	Capital purchase	Dec. 2021	As per the budget allocation for capital purchase, AU$10,000 was paid to each country; Ethiopia, Nepal and Pakistan. There was no allocation of funds for capital purchase for India.

7 Key results and discussion

The project team continued to build genetic resources and information that are beginning to provide valuable insight into the effectiveness of sources of putative durable resistance in protecting wheat crops from stripe rust infection across diverse environments. The ongoing common occurrence and increased severity of stripe rust in wheat crops in recent years in some of our partner countries continues to underscore the relevance and importance of the project. The main focus was to find sources of durable rust resistance that are effective across environments and years, and to begin to address the knowledge gap of why some sources of APR are less effective in some situations. There are a number of notable key achievements from the research from this project:

Field rust screening of 3,000 geographically diverse genotypes during 2017 crop season in Australia, at PBI Cobbitty revealed that 10%, 25%, 35%, 9% and 21% of the genotypes were highly resistant, resistant, moderately resistant, moderately susceptible, and susceptible to stripe rust, respectively (**Fig. 11**).

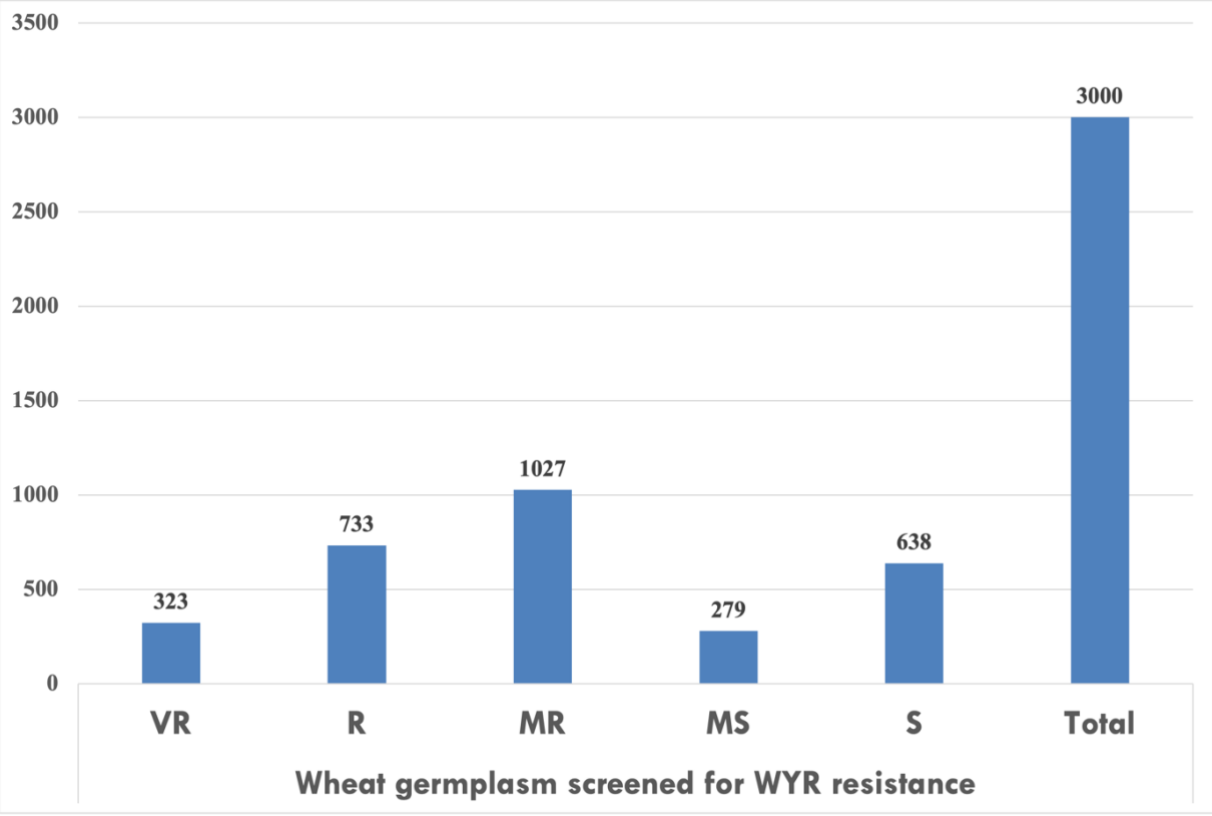

Fig. 11: Field rust screening of germplasm at PBI, Cobbitty, Australia

Core Set

The Core Set of 250 wheat cultivars was selected from rust tests of 3,000 genotypes. The Core Set included 50 well adapted wheat cultivars each from Australia, Ethiopia, India, Kenya, Nepal and Pakistan. Core Set field rust screening across the environments revealed that although most of the genotypes were resistant to *Pst* in Australia, many were vulnerable to *Pst* at field sites along the Himalayan region in SA. Field trials revealed that seedling genes *Yr5*, *Yr10*, *Yr15*, *Yr24*, *Yr26*, *YrSk*, *YrCD* and *YrSp* are still effective in SA, and that virulence is present for *Yr2*, *Yr9* and *Yr17.* Interestingly, 147 Core Set genotypes expressed triple rust resistance under Australian condition, and details of these genotypes are provided in **Appendix X**. These genotypes represent valuable sources of resistance to stripe rust for breeding programs in Australia and all partner countries. Based on the field-testing data, greenhouse multi-pathotype testing (using eight different Australian pathotypes of *Pst*) data and genotyping conducted at PBI, ASR *Yr* resistance genes were postulated in the Core Set genotypes (**Fig. 12**).

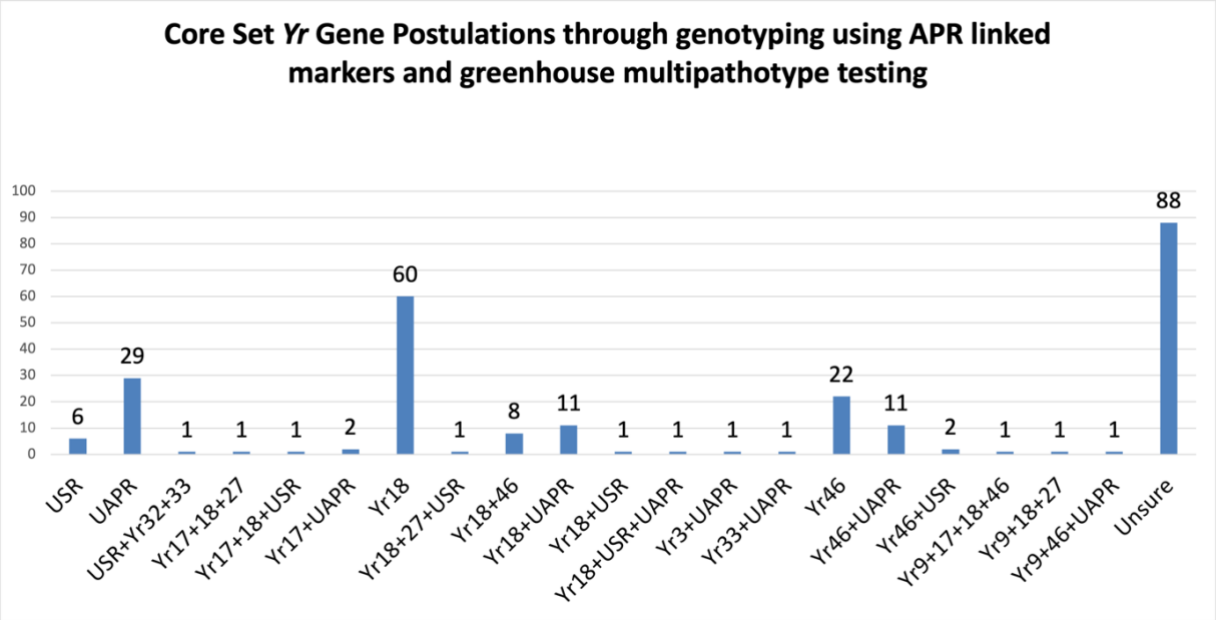

Fig. 12: Postulations of ASR *Yr* genes in the Core Set entries and of known APR genes through genotyping with linked markers

Gene postulations showed that Core Set genotypes are highly diverse in resistance to *Pst* resistance. Genotyping using molecular markers linked to the catalogued stripe rust APR genes *Yr18* (**Fig. 13**), *Yr36* and *Yr46* revealed that 77 and 36 Core Set genotypes carried *Yr18* and *Yr46*, respectively, nine genotypes carried both *Yr18* and *Yr46*, and none of the lines carried *Yr36* (**Fig. 14**).

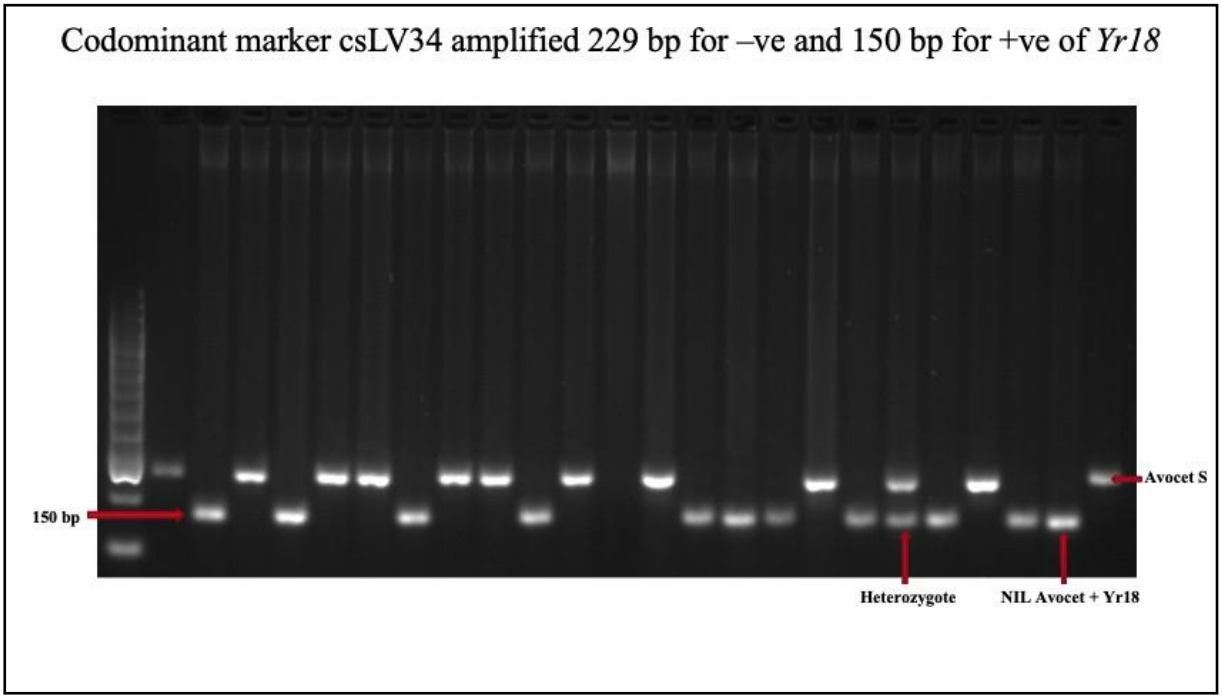

Fig. 13: Core Set genotyping using *Yr18* linked marker csLV34

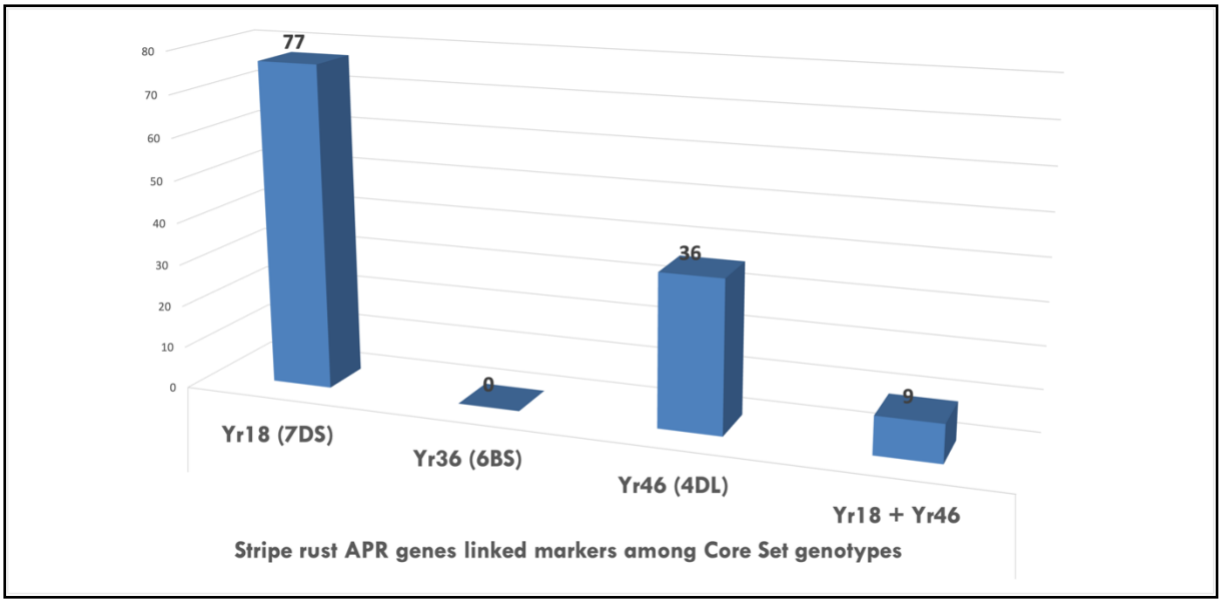

Fig. 14: Detection of stripe rust APR genes linked markers in the Core Set

A combined analysis of multi-environment field rust screening data and greenhouse multi-pathotype testing data revealed 50 of the Core Set genotypes carried APR only. These 50 genotypes (Australia [7], Ethiopia [8], India [17], Nepal [10] and Pakistan [8]) were susceptible to all pathotypes used in the greenhouse multi-pathotype tests but **remained consistently resistant over three years under field conditions in Australia, SA and EA.** According to the analysis in conjunction with the genotyping data, 29 of these 50 genotypes

carried unidentified APR (UAPR), 10 carried *Yr18*+UAPR, 10 carried *Yr46*+UAPR and one carried the combination of *Yr18*+*Yr*46+UAPR (**Fig. 15**).

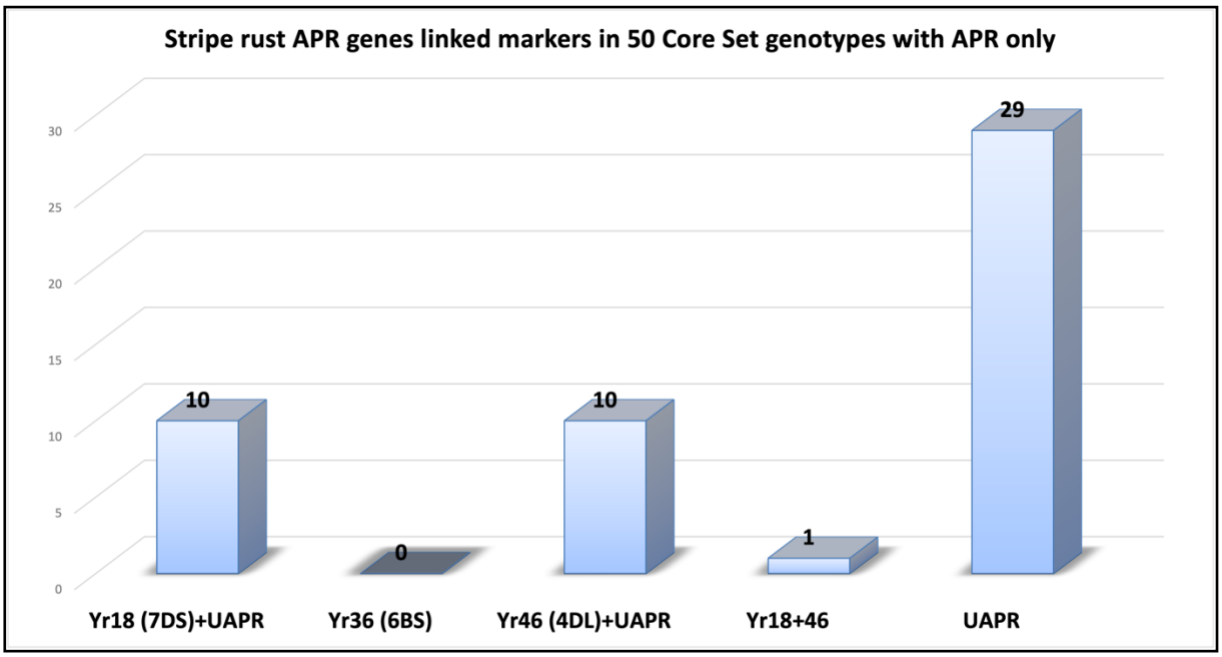

Fig. 15: Stripe rust APR genes linked markers in the 50 Core Set genotypes carrying APR only

The Core Set was also genotyped using the 90Kiselect SNP chip, and this data was used to dissect the genetic basis of stripe rust resistance among the 250 genotypes. The Core Set GWAS revealed the presence of 20 significant QTL on chromosomes 2A, 3A, 5A, 6A, 7A, 1B, 2B, 4B, 5B and 7B (**Table 2**, **Figs 16** and **17**), and no QTL was detected on any D genome chromosome (**Fig. 18**). Interestingly, 18 of the 20 QTL detected in the Core Set are present in the 50 genotypes characterised to carry APR only. The greater number of markers contributing to the QTL provided high confidence in the assignments in the corresponding genotypes. Apart from six QTL (QTL3-2A-AUS, QTL8-5A-AUS, QTL10-7A-AUS, QTL12-1B-AUS, QTL16-4B-ETH and QTL17-4B-ETH), all are associated with more than three markers providing confidence in the assignments. Further Manhattan Plots (**Figs 19a-f**) using "ggplot 2" (Wickham 2016), showing the association of at least three markers within 20Mbp of resistance validated the significant associations at >4 LOD score with the QTL assigned. In total, 47 unique QTL combinations were detected in the Core Set entries.

Given further funding, these QTL could be validated and fine mapped for marker development so that could be rapidly introgressed into high yielding backgrounds using marker assisted selection of the APR genes identified.

Table 2: Details of QTL detected for stripe rust resistance in the 250 Core Set genotypes, 50 of which carried APR only

QTL name	Chr	Peak marker	No. markers	Frequency in Core Set	Frequency in APR Panel
QTL1-2A-PAK	2A	Kukri_c4698_3401	9	9%	0%
QTL2-2A-AUS	2A	RAC875_rep_c88665_52	12	11%	28%
QTL3-2A-AUS	2A	RAC875_c20247_398	3	89%	74%
QTL4-3A-PAK	3A	TA005199-0585	8	9%	16%
QTL5-3A-PAK	3A	wsnp_Ex_rep_c69567_68516173	5	90%	94%
QTL6-3A-PAK	3A	IAAV5043	6	88%	96%
QTL7-3A-AUS	3A	Jagger_c7131_109	4	46%	44%
QTL8-5A-AUS	5A	TA005221-1244	3	8%	10%
QTL9-6A-ETH	6A	GENE-3948_357	22	60%	54%
QTL10-7A-AUS	7A	Tdurum_contig11613_329	3	55%	52%
QTL11-1B-NPL	1B	RAC875_c63624_1040	14	45%	44%
QTL12-1B-AUS	1B	Ku_c8505_1424	3	46%	54%
QTL13-1B-NPL	1B	RAC875_c14902_256	9	22%	0%
QTL14-1B-NPL	1B	BS00050457_51	14	6%	4%
QTL15-2B-KEN	2B	Ra_c1501_1220	6	23%	24%
QTL16-4B-ETH	4B	Excalibur_c38108_290	3	83%	96%
QTL17-4B-ETH	4B	RAC875_c36213_352	3	83%	70%
QTL18-5B-ETH	5B	GENE-3619_539	9	65%	64%
QTL19-5B-ETH	5B	TA003709-0269	12	63%	50%
QTL20-7B-NPL	7B	tplb0040b02_1219	5	34%	26%

GWAS also revealed a significant phenotypic rust response correlation between the environments in SA (**Fig. 20**), showing the resistance identified was in many cases effective across environments. These correlations did however show that many resistances performed poorly at Meraro (Ethiopia). This is most likely due to differences in pathogen virulence, environment, or both.

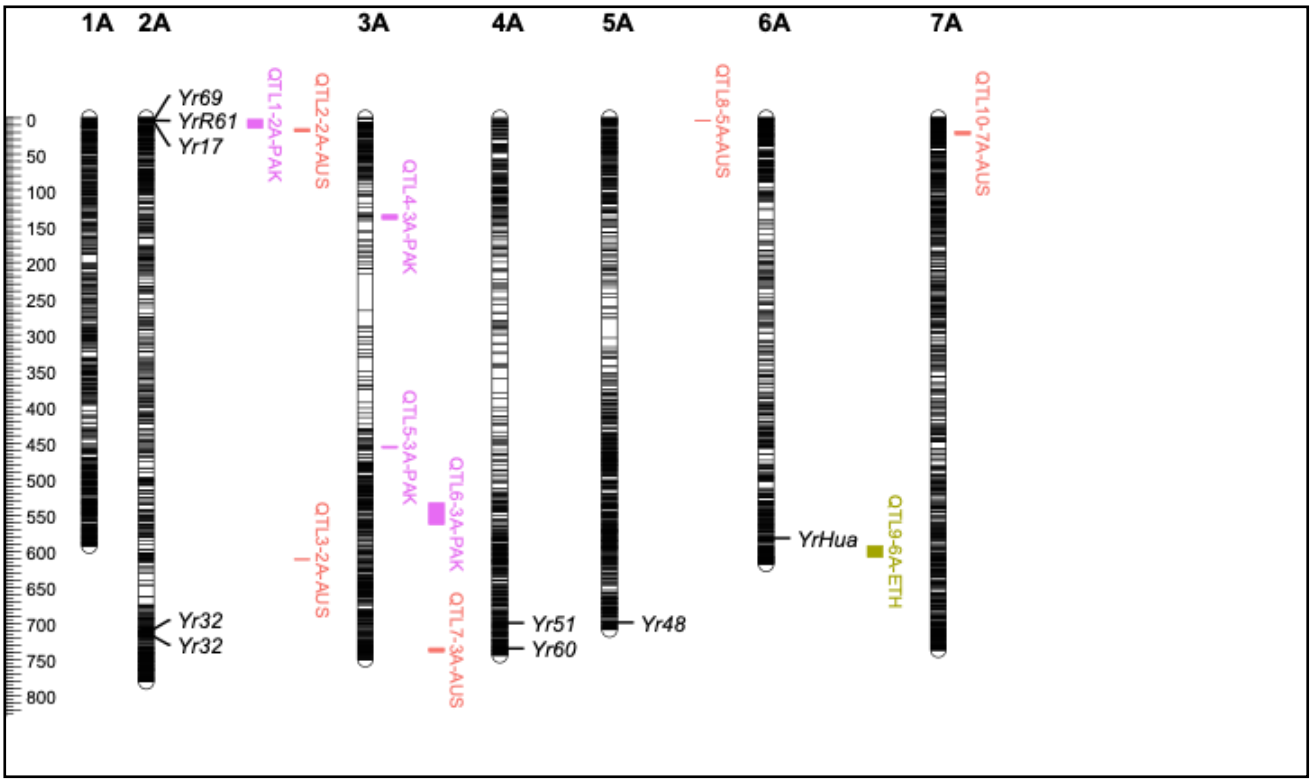

Fig. 16: 10 significant QTL for APR to stripe rust on A genome chromosomes detected in GWAS analyses

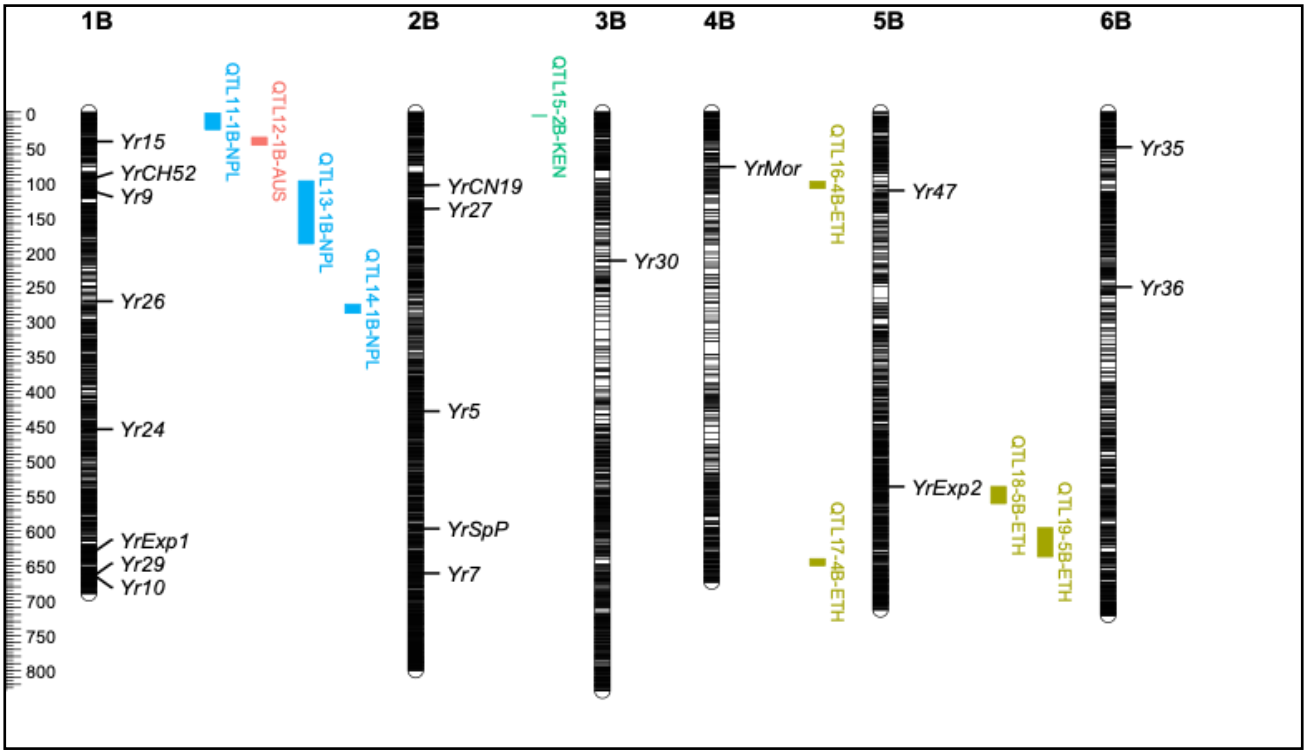

Fig. 17: 10 significant QTL for APR to stripe rust on B genome chromosomes detected in GWAS analyses

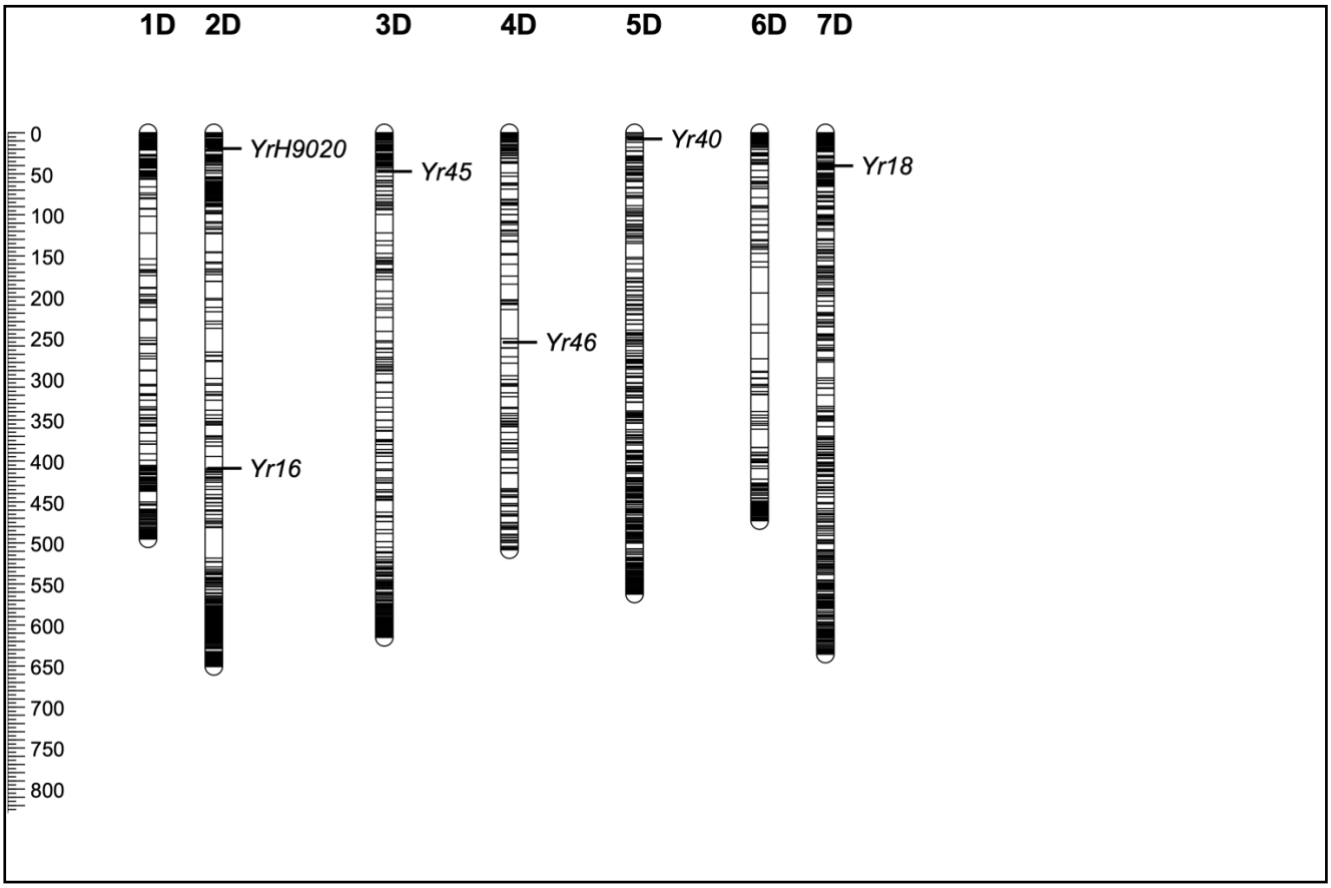

Fig. 18: No significant QTL were detected on D genome chromosomes in the GWAS analyses

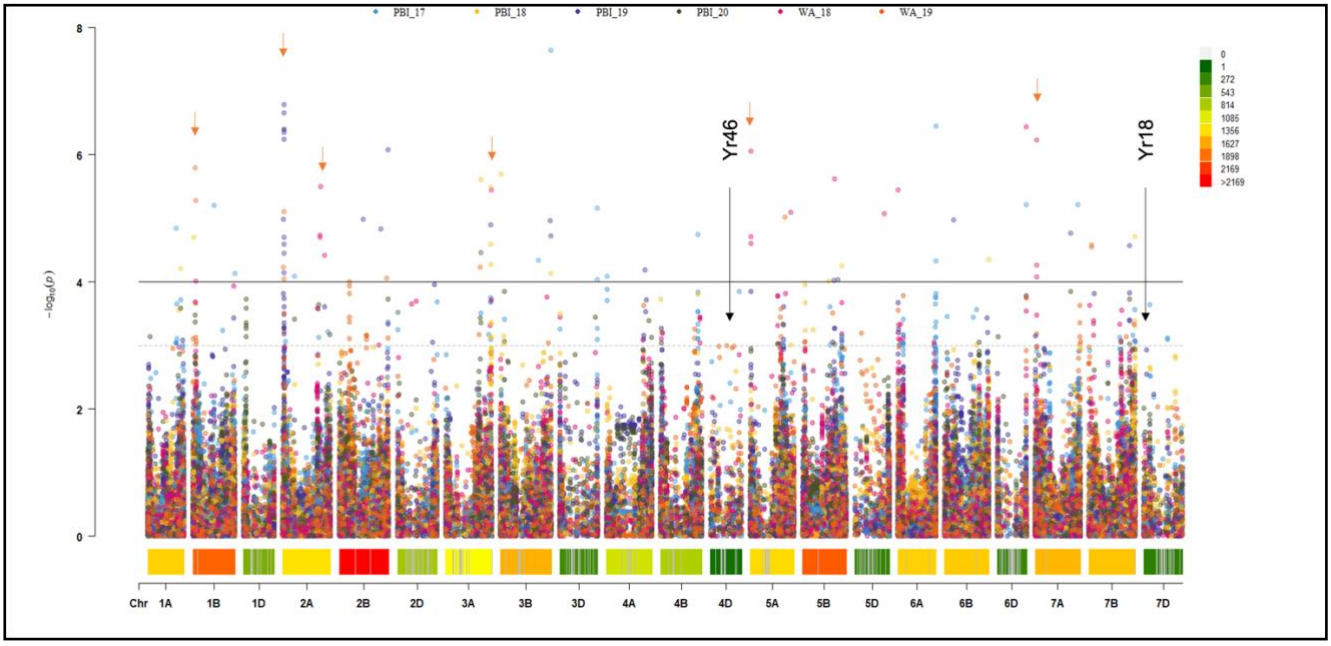

Fig. 19a: Manhattan plot showing significant QTL (indicated with orange arrows) associated with stripe rust resistance at >4 LOD score among the Core Set entries, tests conducted in Australia

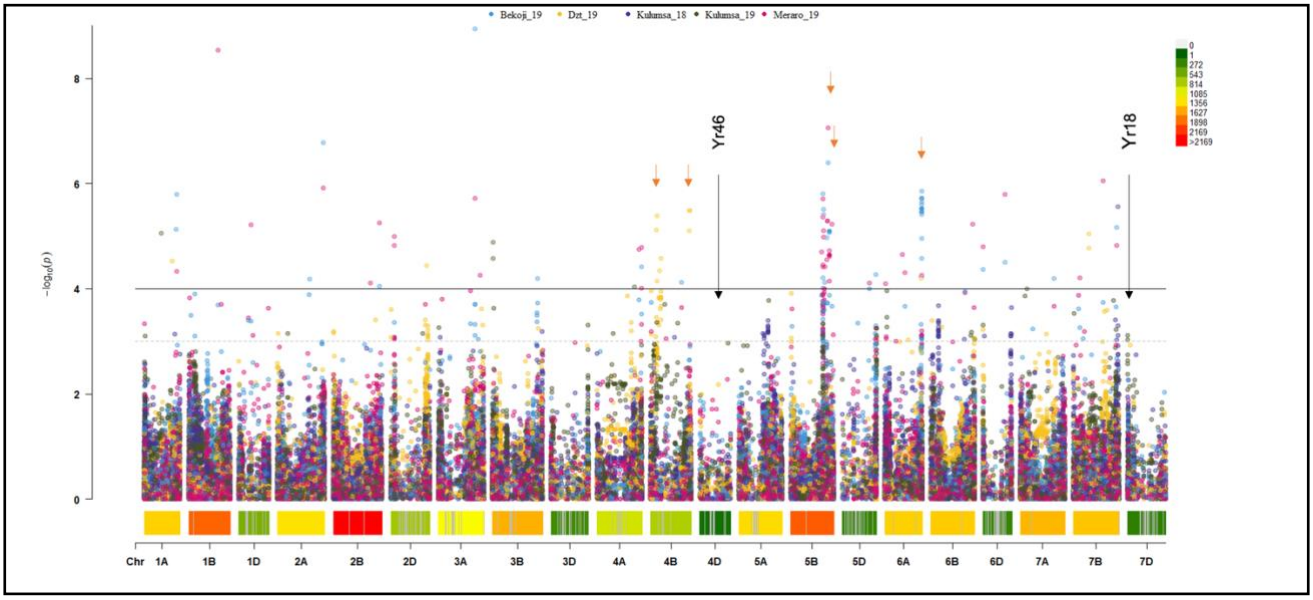

Fig. 19b: Manhattan plot showing significant QTL (indicated with orange arrows) associated with stripe rust resistance at >4 LOD score among the Core Set entries, tests conducted in Ethiopia

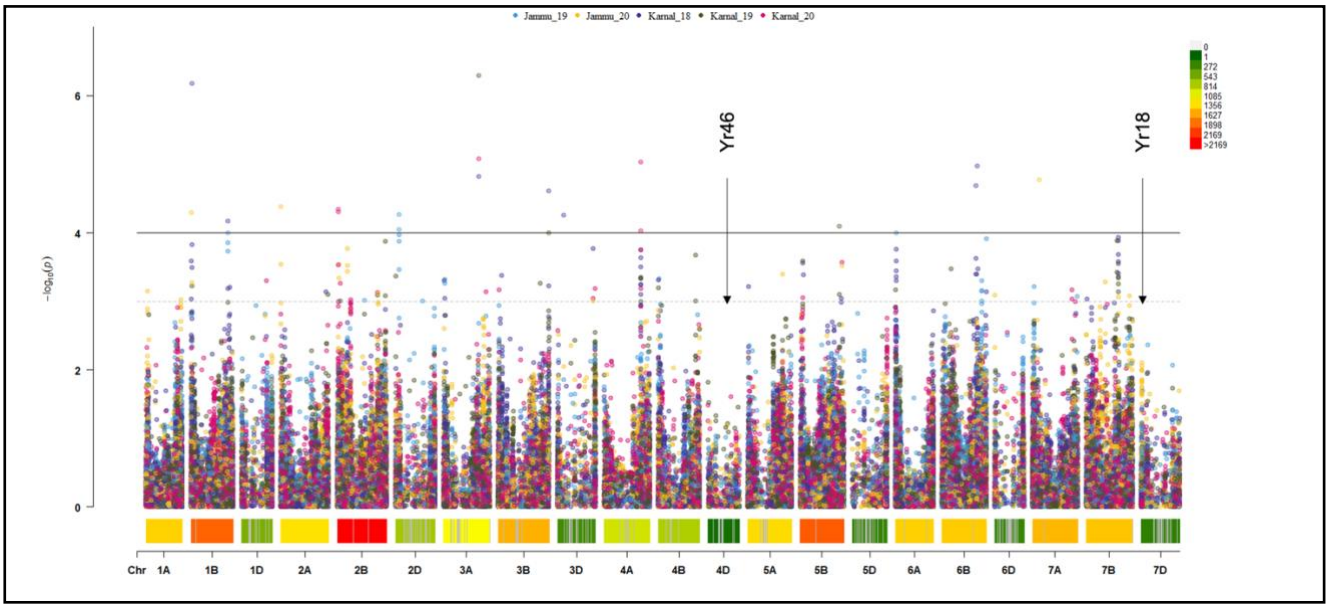

Fig. 19c: Manhattan plot showing significant QTL (indicated with orange arrows) associated with stripe rust resistance at >4 LOD score among the Core Set entries, tests conducted in India

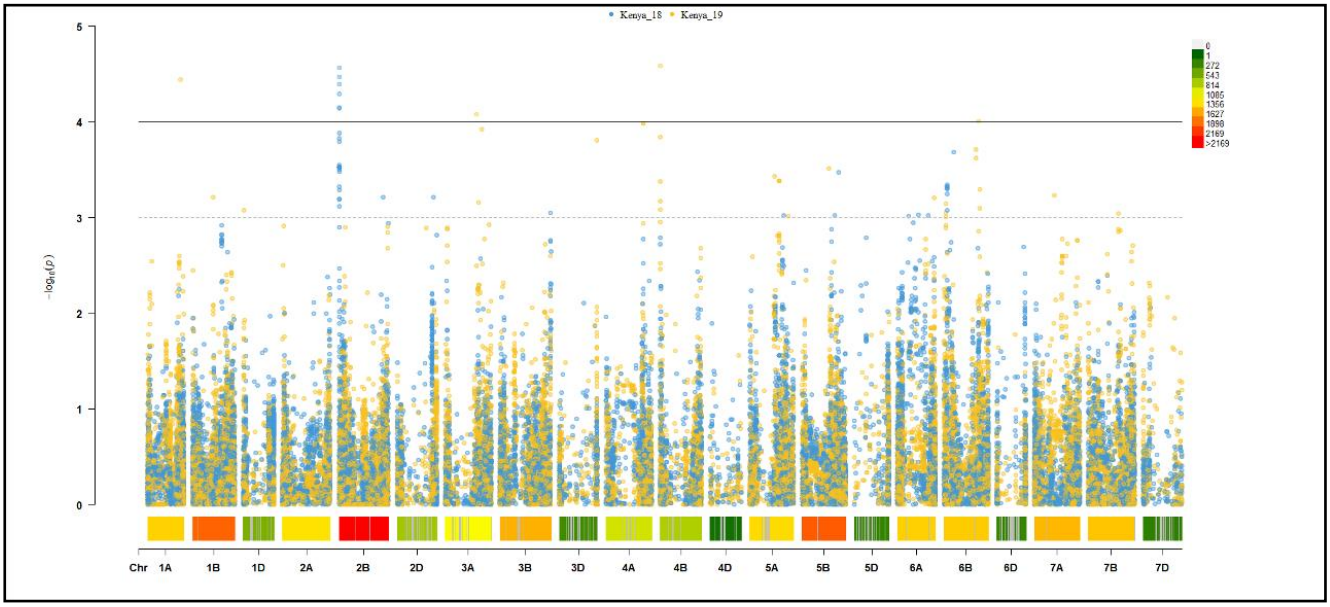

Fig. 19d: Manhattan plot showing significant QTL (indicated with orange arrows) associated with stripe rust resistance at >4 LOD score among the Core Set entries, tests conducted in Kenya

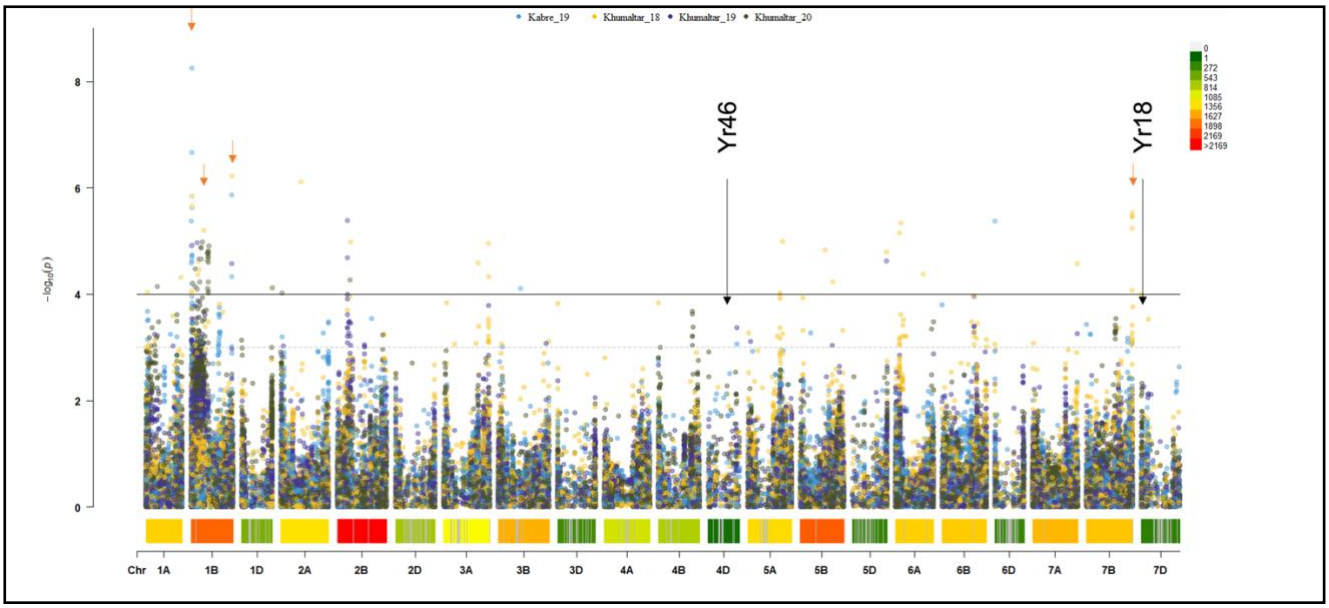

Fig. 19e: Manhattan plot showing significant QTL (indicated with orange arrows) associated with stripe rust resistance at >4 LOD score among Core Set entries, tests conducted in Nepal

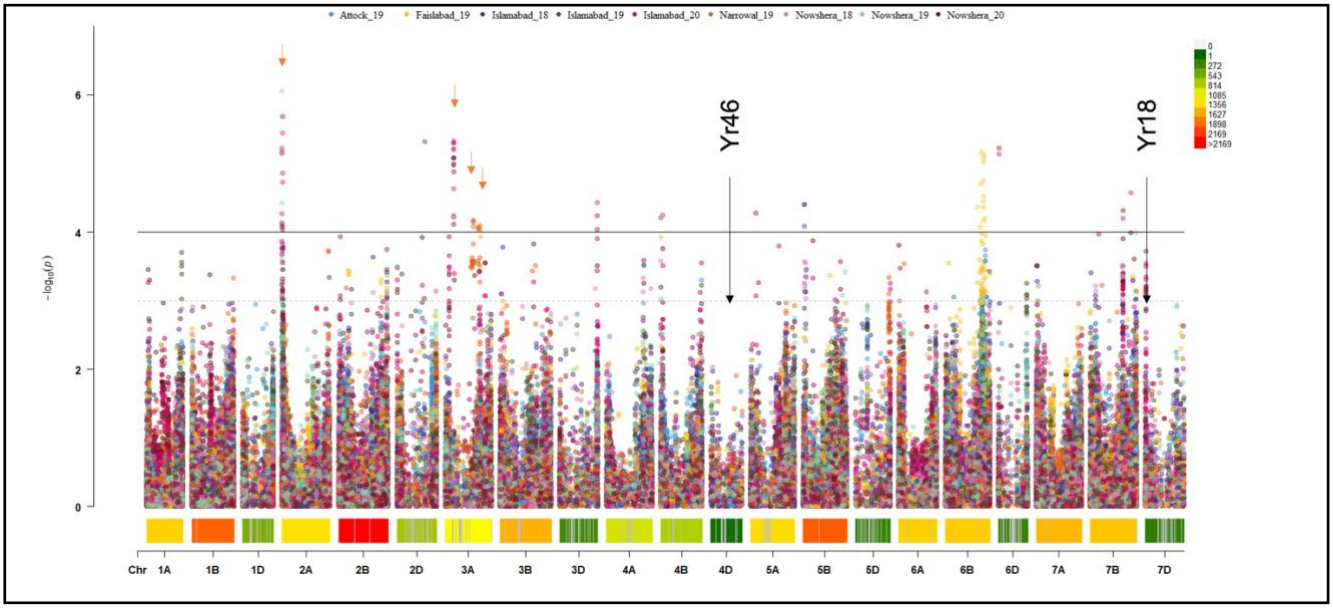

Fig. 19f: Manhattan plot showing significant QTL (indicated with orange arrows) associated with stripe rust resistance at >4 LOD score among Core Set entries, tests conducted in Pakistan

		Australia					Ethiopia					India					Nepal				Pakistan									Kenya	
		PBI_18	PBI_19	PBI_20	WA_18	WA_19	Bekoji_19	Dzt_19	Kulumsa_18	Kulumsa_19	Meraro_19	Jammu_19	Jammu_20	Karnal_18	Karnal_19	Karnal_20	Kabre_19	Khumaltar_18	Khumaltar_19	Khumaltar_20	Attock_19	Faislabad_19	Islamabad_18	Islamabad_19	Islamabad_20	Narrowal_19	Nowshera_18	Nowshera_19	Nowshera_20	Kenya_18	Kenya_19
Australia	PBI_17	0.64***	0.46***	0.47***	0.52***	0.48***	0.00	-0.04	0.42***	0.35***	-0.02	0.27***	0.25***	0.38***	0.45***	0.26***	0.2**	0.47	0.47***	0.38***	0.3***	0.19**	0.36***	0.31***	0.14*	0.14*	0.36***	0.3***	0.14*	0.39***	0.57***
	PBI_18		0.49***	0.57***	0.53***	0.63***	-0.10	-0.01	0.46***	0.27***	-0.11	0.39***	0.34***	0.5***	0.57***	0.4***	0.44***	0.6***	0.54***	0.33***	0.4***	0.44***	0.59***	0.48***	0.24***	0.28***	0.57***	0.43***	0.23***	0.45***	0.47***
	PBI_19			0.59***	0.53***	0.27***	0.01	-0.02	0.29***	0.24***	-0.01	0.14*	0.17**	0.37***	0.35***	0.27***	0.5***	0.45***	0.35***	0.25***	0.24***	0.19**	0.3***	0.25***	0.13*	0.08	0.32***	0.24***	0.10	0.35***	0.4***
	PBI_20				0.44***	0.34***	-0.09	-0.02	0.39***	0.33***	-0.08	0.32***	0.33***	0.39***	0.45***	0.41***	0.45***	0.54***	0.48***	0.34***	0.3***	0.33***	0.51***	0.4***	0.27***	0.12*	0.53***	0.39***	0.24***	0.54***	0.38***
	WA_18					0.61***	-0.04	-0.03	0.33***	0.12*	-0.04	0.19**	0.11*	0.28***	0.34***	0.23***	0.35***	0.36***	0.33***	0.27***	0.2**	0.09	0.29***	0.24***	0.13*	0.13*	0.3***	0.24***	0.12*	0.34***	0.38***
	WA_19						-0.05	-0.02	0.34***	0.12*	-0.07	0.26***	0.2**	0.35***	0.31***	0.29***	0.23***	0.45***	0.46***	0.28***	0.25***	0.29***	0.4***	0.33***	0.22***	0.23***	0.38***	0.31***	0.21**	0.28***	0.29***
Ethiopia	Bekoji_19						NA	0.53***	0.27**	0.9***		-0.14	0.08	-0.13	0.02	-0.02	-0.07	-0.04	0.2*	0.2*	-0.13	-0.03	-0.06	-0.13	0.05	-0.04	-0.06	-0.13	0.05	-0.09	0.04
	Dzt_19							0.07	0.19**	NA		-0.02	-0.03	0.04	-0.03	-0.02	-0.03	0.02	0.02	-0.02	-0.01	-0.04	0.03	-0.03	-0.02	-0.05	0.00	-0.04	0.00	-0.03	0.02
	Kulumsa_18								0.61***	0.36***		0.39***	0.39***	0.48***	0.48***	0.37***	0.34***	0.58***	0.55***	0.34***	0.43***	0.48***	0.54***	0.53***	0.3***	0.18**	0.5***	0.51***	0.27***	0.69***	0.67***
	Kulumsa_19									0.24*		0.11	0.11	0.10	0.2**	0.12*	0.36***	0.36***	0.44***	0.34***	0.15*	0.16*	0.11	0.2**	0.21**	-0.06	0.11	0.18*	0.18*	0.36***	0.46***
	Meraro_19											-0.13	0.05	-0.14	0.05	-0.06	-0.07	-0.06	0.13	0.18*	-0.15	-0.07	-0.07	-0.16	0.00	-0.06	-0.08	-0.15	0.01	-0.08	0.03
India	Jammu_19												0.59**	0.65***	0.55***	0.63***	0.18**	0.32***	0.27***	0.09	0.72***	0.59***	0.64***	0.75***	0.43***	0.32***	0.59***	0.73***	0.4***	0.47***	0.25***
	Jammu_20													0.48***	0.46***	0.48***	0.2**	0.35***	0.3***	0.15*	0.5***	0.54***	0.67***	0.6***	0.4***	0.23***	0.64***	0.58***	0.37***	0.4***	0.2**
	Karnal_18														0.57***	0.62***	0.31***	0.47***	0.36***	0.18**	0.58***	0.61***	0.7***	0.63***	0.32***	0.32***	0.68***	0.62***	0.28***	0.47***	0.45***
	Karnal_19															0.56***	0.33***	0.44***	0.35***	0.19**	0.48***	0.51***	0.64***	0.56***	0.32***	0.3***	***	0.51***	0.3***	0.52***	0.36***
	Karnal_20																0.27***	0.38***	0.28***	0.15*	0.61***	0.53***	0.63***	0.66***	0.34***	0.28***	0.65***	0.64***	0.32***	0.49***	0.27***
Nepal	Kabre_19																	0.58***	0.47***	0.28***	0.17**	0.25***	0.31***	0.24***	0.11*	-0.01	0.32***	0.24***	0.10	0.33***	0.31***
	Khumaltar_18																		0.87***	0.51***	0.31***	0.46***	0.53***	0.44***	0.21**	0.23***	0.54***	0.43***	0.2**	0.48***	0.48***
	Khumaltar_19																			0.68***	0.28***	0.39***	0.41***	0.38***	0.23***	0.15*	0.41***	0.39***	0.22***	0.41***	0.38***
	Khumaltar_20																				0.15*	0.22***	0.23***	0.19**	0.17**	0.07	0.23***	0.2**	0.15*	0.2**	0.21**
Pakistan	Attock_19																					0.63***	0.63***	0.86***	0.51***	0.31***	0.6***	0.84***	0.48***	0.48***	0.25***
	Faislabad_19																						0.7***	0.71***	0.37***	0.45***	0.69***	0.69***	0.34***	0.51***	0.33***
	Islamabad_18																							0.73***	0.42***	0.38***	0.95***	0.69***	0.39***	0.57***	0.38***
	Islamabad_19																								0.57***	0.34***	0.68***	0.91***	0.54***	0.57***	0.34***
	Islamabad_20																									0.19**	0.38***	0.58***	0.96***	0.31***	0.18*
	Narrowal_19																										0.38***	0.35***	0.18**	0.13*	0.09
	Nowshera_18																											0.65***	0.35***	0.55***	0.36***
	Nowshera_19																												0.53***	0.54***	0.34***
	Nowshera_20																													0.28***	0.15*
Kenya	Kenya_18																														0.58***

*** significance at the 0.1% level
** significance at the 1.0% level
* significance at the 5.0% level

Fig. 20: Phenotypic correlations between resistance levels expressed between environments and within the countries

Yellow spot resistance in Core Set genotypes

Most of the Core Set genotypes were resistant to YLS when tested under field conditions in Western Australia. Of the 250 genotypes, 8 were R, 8 RMR, 37 MR, 59 MRMS and 74 MS and only 15, 6 and 2 were S, SVS and VS to YLS respectively (**Fig. 21**). In addition to being a source of resistance to WYR, the Core Set entries are also clearly a valuable resource of resistance to YLS.

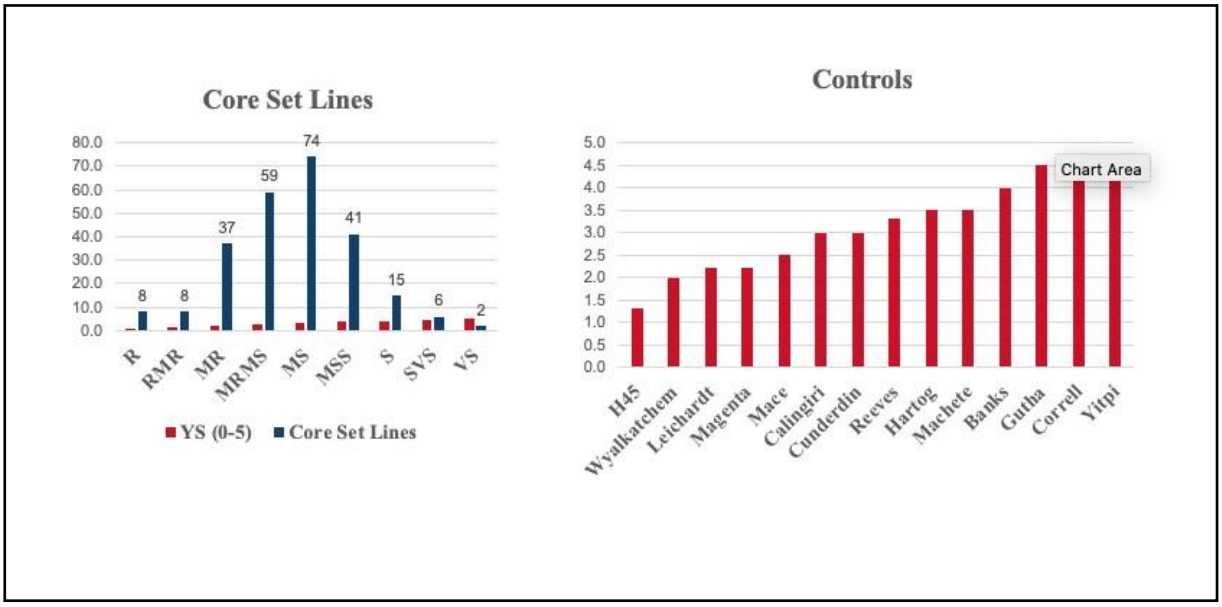

Fig. 21: Response of the Core Set entries and control genotypes to YLS in field tests conducted in WA

Synthetic wheat genotypes

Out of 766 synthetic wheat genotypes tested during the 2016 and 2017 crop seasons, 591 were consistently resistant to WYR in Australia. A subset of 94 non-lodging genotypes with unique pedigrees (**Appendix III**) was established from these 591 genotypes. These 94 genotypes were further tested under field conditions consecutively for three seasons in Australia and at least for one crop season in Ethiopia, India, Kenya, Nepal and Pakistan. Under Australian field conditions, most of the genotypes were highly resistant (VR–RMR), except two genotypes (AUS30623 and AUS33408), which exhibited MR–MS (score 4–6 as per the 1–9 scale). Out of 94 genotypes, 92, 30, 63, 74, 48 and 32 genotypes were characterised with high levels ((score 1–3 (VR–RMR)) of *Pst* resistance when tested under field conditions of Australia, Ethiopia, India, Kenya, Nepal and Pakistan respectively (Fig. 27). Similarly, 2, 49, 31, 14, 17 and 11 genotypes expressed medium levels (score 4–6) of *Pst* resistance under field conditions of Australia, Ethiopia, India, Kenya, Nepal and Pakistan respectively (**Fig. 22**). Numbers of genotypes that expressed low levels (score 7) of *Pst* resistance under field conditions, were more in Nepal and Pakistan, as compared the field expressions recorded from the number of genotypes in Ethiopia (Kulumsa) and Kenya (**Fig.**

22). None of the line expressed low levels of resistance under field conditions of Australia and India.

Eight genotypes (AUS30265, AUS30266, AUS30268, AUS30282, AUS30283, AUS30288, AUS30320 and AUS30505) were identified as being VR–RMR (1–3) and 17 genotypes (AUS30269, AUS30273, AUS30279, AUS30336, AUS30341, AUS30353, AUS30538, AUS30552, AUS30615, AUS30617, AUS30635, AUS33388, AUS34097, AUS34116, AUS34142, AUS34198, AUS34219) as MR–MS (4–6) across all environments in Australia, EA, and SA.

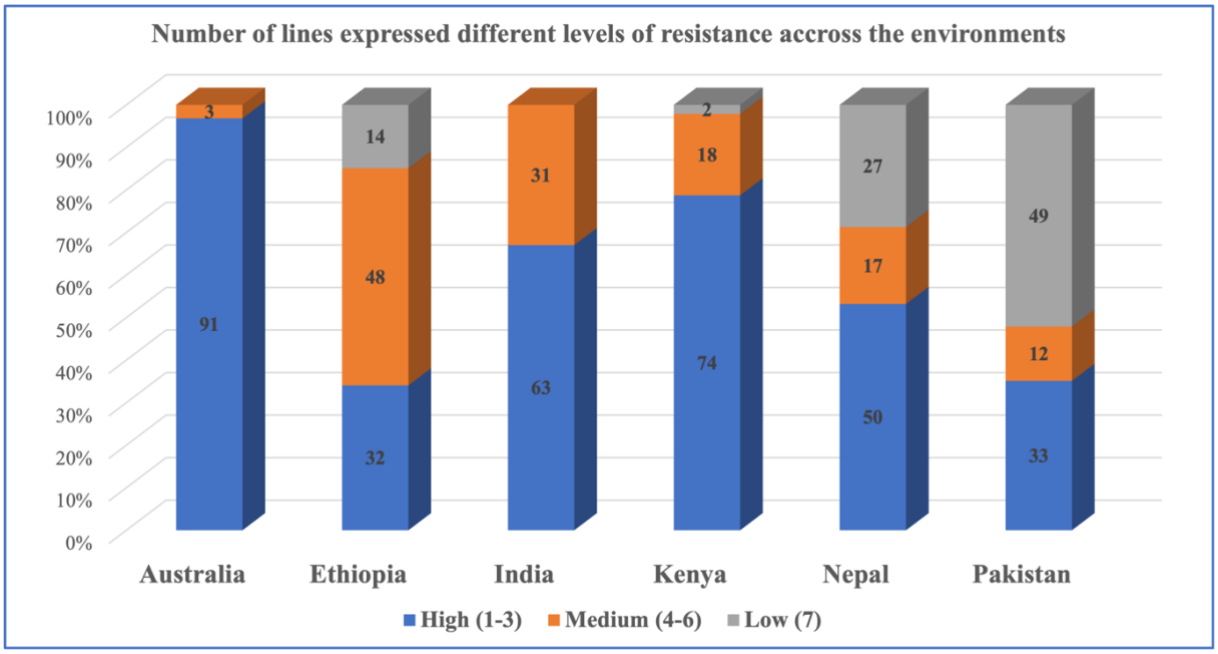

Fig. 22: Rust responses of 94 synthetic wheat genotypes tested across the environments

In Australia, consistently for five crop seasons, 97% of the synthetic wheat genotypes were scored as 1–3 (R–MR) and only 3% of the genotypes showed the rust score of 5–6 (MRMS–MS) when tested under field conditions. Based on the field rust screening results, greenhouse multi-pathotype testing using nine Australian Pts of *Pst* (**Appendix IV**) and genotyping with APR genes linked markers (**Appendix VII**), seven categories (**Fig. 28**) of response to *Pst* were identified among the 94 synthetic genotypes. Postulated resistance genes included UAPR, 11%, USR, 46%, known seedling resistance (KSR, 5%), KSR + USR (2%), *Yr18* + UAPR (4%), *Yr18* + USR (29%) and *Yr18* + KSR (3%) (**Fig. 23**).

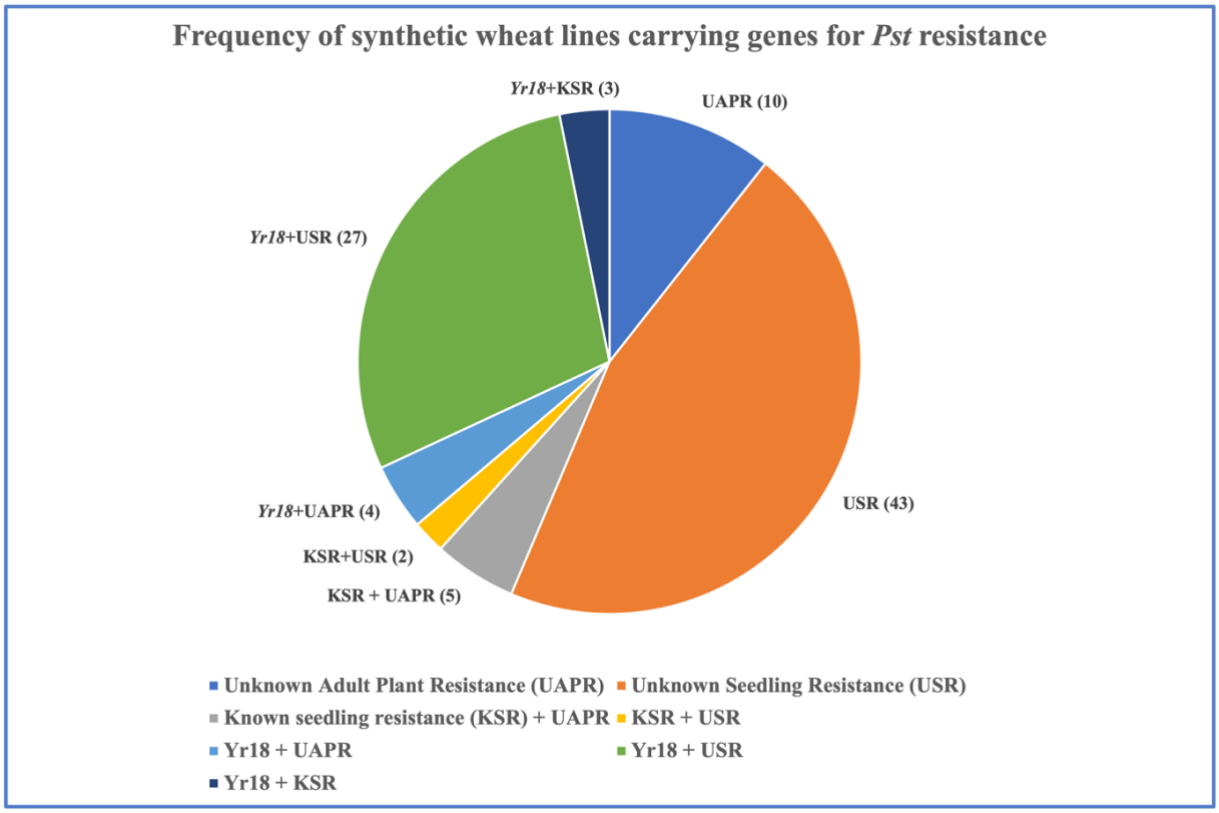

Fig. 23: Gene postulations in synthetic wheat genotypes through rust screening both in the greenhouse and under field conditions and genotyping using markers linked with APR genes

All genotypes were tested at the seedling stage using nine different Australian pathotypes of *Pst* in the greenhouse. Multi-pathotype testing revealed that 14 genotypes (AUS30288, AUS30332, AUS30509, AUS30515, AUS30577, AUS33403, AUS33406, AUS34091, AUS34097, AUS34148, AUS30619, AUS33376, AUS33381, AUS34169) lacked any seedling gene for *Pst* resistance. These 14 genotypes were resistant when tested under field conditions across the environments and were thus characterised to carry UAPR. Genotyping revealed that four genotypes (AUS30619, AUS33376, AUS33381, AUS34169) also *Yr18* in addition to the UAPR. Genotypes AUS30288, AUS30515 and AUS34097 were exceptionally more resistant as compared to the other genotypes characterised with UAPR. Susceptible control genotype Avocet S was rated as 9 (1-9 scale) and *Yr18* (NIL AvocetS+*Yr18*) was scored 7 across all the field environments of Australia, EA and SA. Molecular analysis showed the presence of APR gene *Yr18* linked marker (csLV34) in 34 genotypes, but none of the genotypes showed the presence of markers linked with APR genes *Yr36* and *Yr46* (**Fig. 24**).

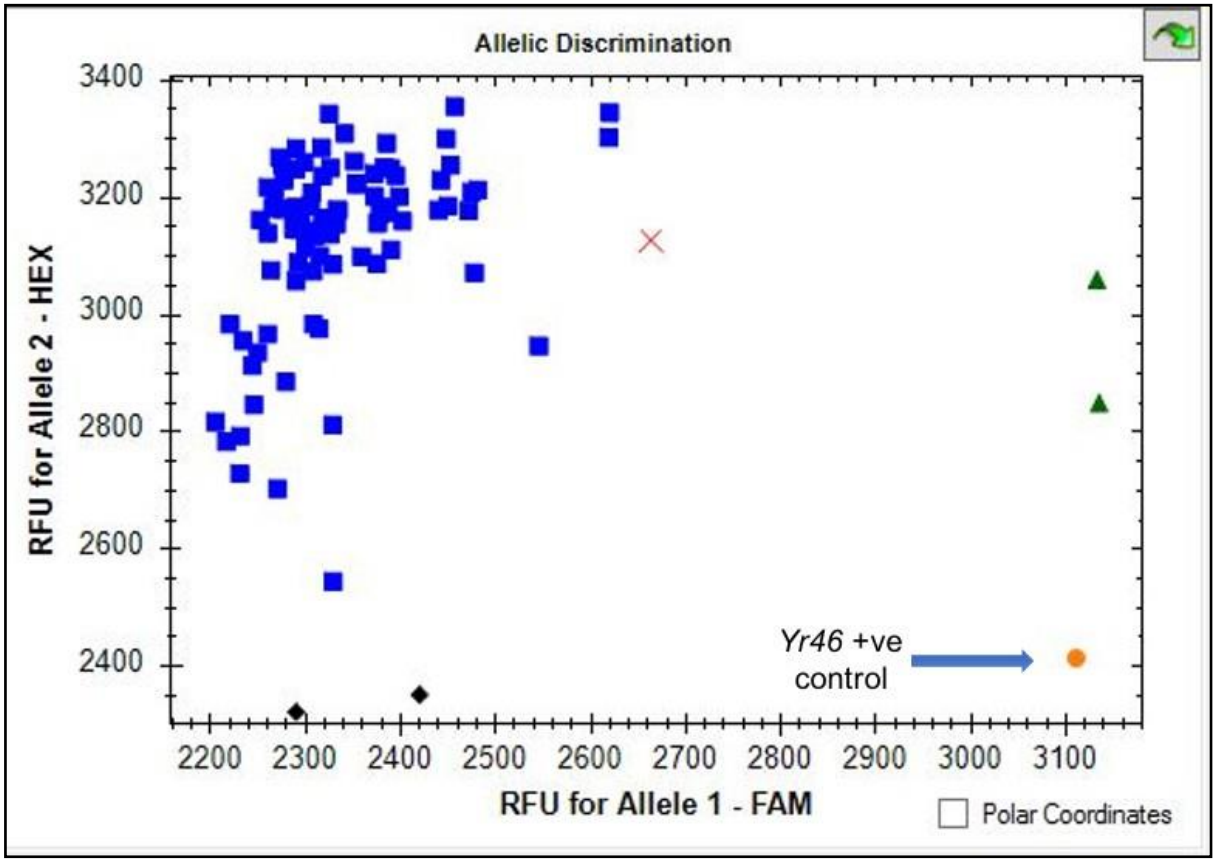

Fig. 24: Genotyping of synthetic wheat genotypes using KASP marker TM4 linked with stripe rust APR gene *Yr46*

Additional wheat germplasm selected

An additional set of germplasm with resistance to *Pst* comprising 300 old wheat genotypes and cultivars was also selected from the field rust screening of 3,000 wheat genotypes. In Australia, out of these selected 300 wheat genotypes, 16, 52, 61, 109, 28, 34 expressed VR, R, RMR, MR, MRMS and MS rust response when tested under field conditions (**Fig. 25**).

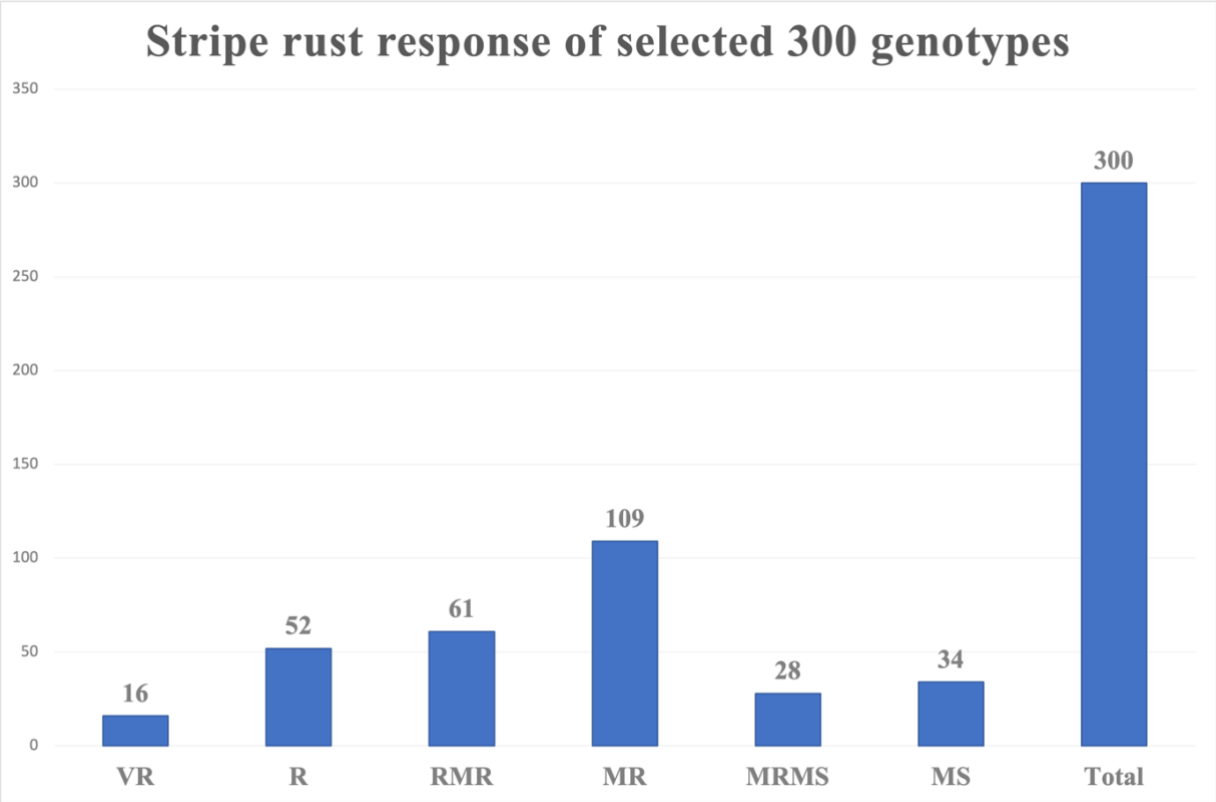

Fig. 25: Rust response of additional germplasm selected for germplasm enhancement

In addition to WYR, data was also recorded for WLR and WSR resistance in Australia, and 127 genotypes out of 300 tested expressed resistance against all three wheat rust diseases. Different levels of resistance expressed against each disease by the number of genotypes is shown in **Fig. 26**.

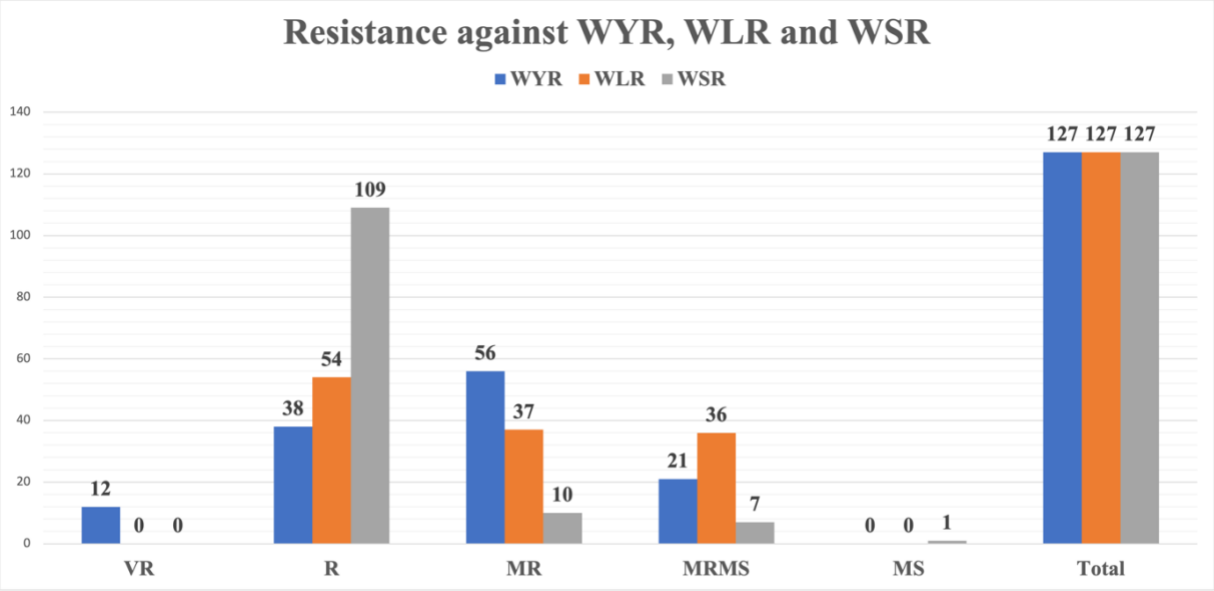

Fig. 26: Triple rust resistance expressed by 127 selected wheat genotypes

Although old and of generally poor agronomic type, these 127 genotypes can be used as donors of triple rust resistance in breeding.

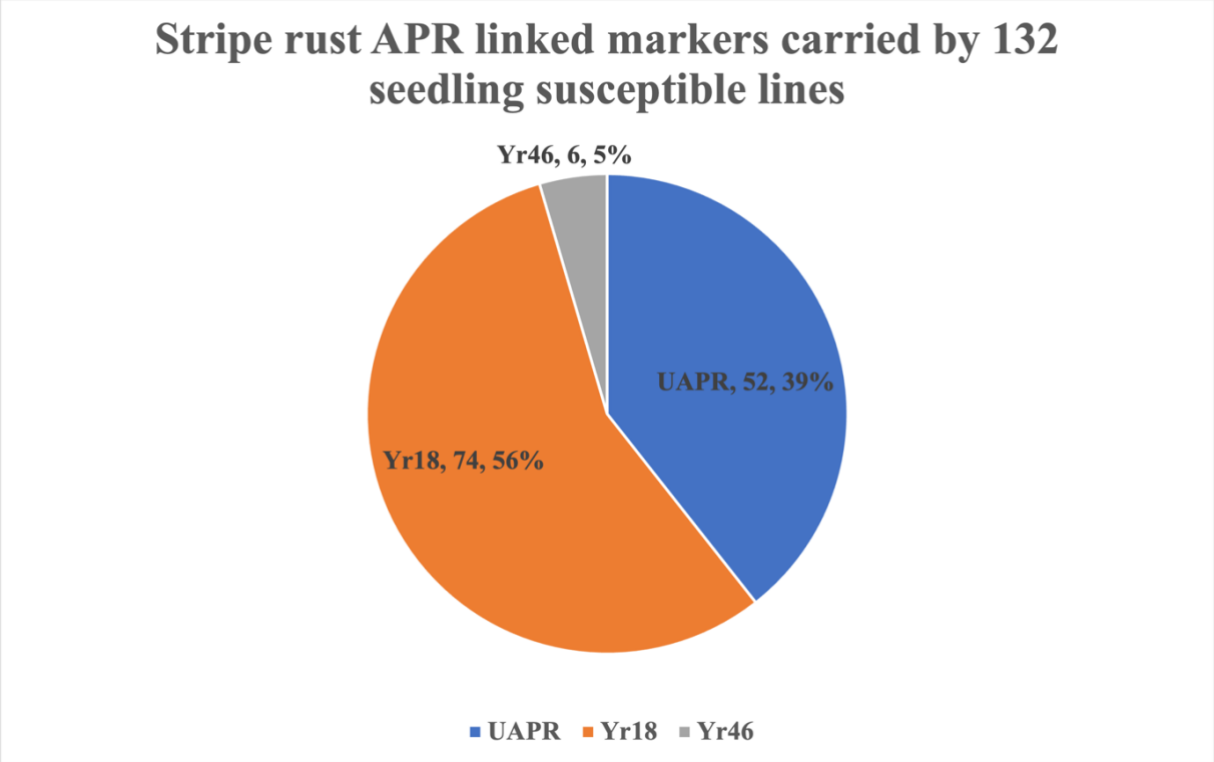

Fig. 27: APR linked markers genotyping of seedling susceptible and field resistant genotypes

Of the 300 genotypes, 132 were susceptible at the seedling stage when tested against the Australian pathotypes of *Pst* but were resistant under field conditions indicating the presence of APR in each. All were genotyped using markers linked to known APR genes, revealing that 56% carried *Yr18*, 5% carried *Yr46*, with the remaining 39% lacking either gene and therefore carrying UAPR (**Fig. 27**).

Nested Association Mapping (NAM) population

Although more than 2,200 BC_1F_5 NAM population RLs were developed, the population size was reduced and a total of 1,452 lines were selected from different 28 crosses for sequencing, multi-pathotype testing and for field rust evaluations across the environments of Australia, EA and SA. Most of the NAM population RLs were characterised as VR to MR for stripe rust resistance in Australia, whereas in Ethiopia most of the lines were MR–MS to MS–S. The breakdown in response of the NAM population RLs to stripe rust resistance in all countries is shown in **Fig. 28** and **Table 3**.

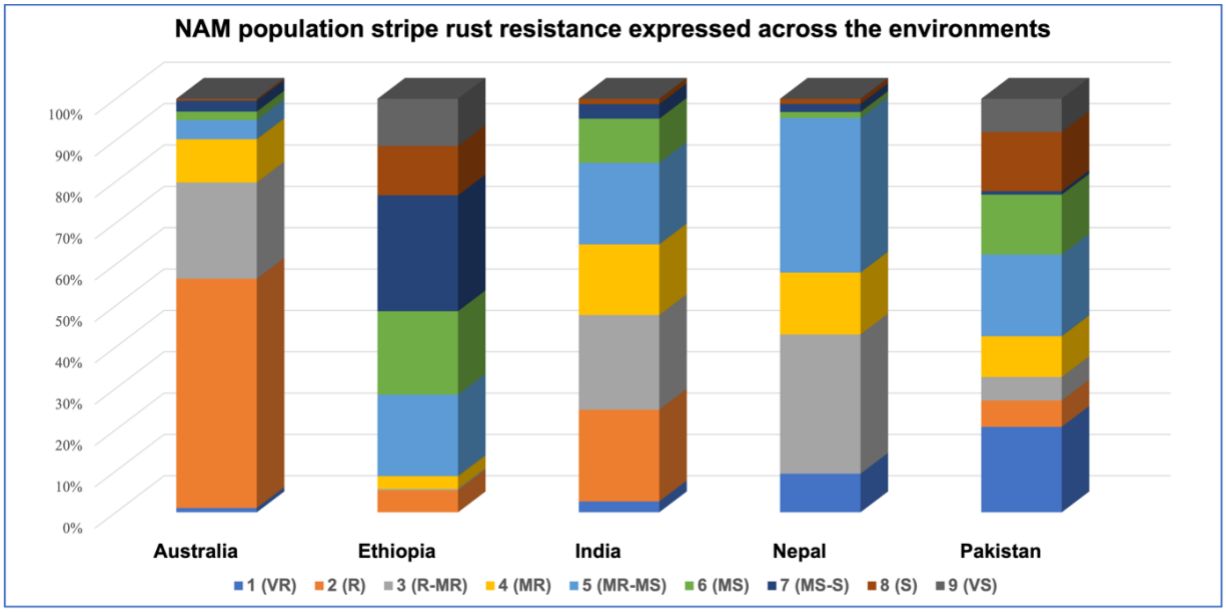

Fig. 28: NAM recombinants characterised for WYR resistance across the environments

Multi-pathotype testing of the 1,452 NAM RLs at the seedling growth stage revealed that 127 were susceptible to all the Australian pathotypes of *Pst* used but were resistant when tested under field conditions in Australia. These 127 RLs are considered to carry APR only, which was highly effective in Australia because most were rated VR to MR (**Appendix XI**). Further, 49 of these 127 RLs remained resistant under field conditions in Ethiopia, India, Nepal and Pakistan. The 49 NAM population RLs (**Appendix XII**) represent eight different crosses (HD2733 x ISR 1043.15 [NAM 9.32], Danphe x Diamondbird [NAM 22.23], Picaflor x Synthetic, AUS30515 [NAM 24.9], Picaflor x ISR 1043.15 [NAM 25.1], Picaflor x Kingbird [NAM 26.20], Picaflor x Diamondbird [NAM 28.1], Gautam x Kingbird [NAM 30.13] and NL 971 x Kingbird [NAM 32.17]), and consequently are presumed to carry high genetic diversity for APR to WYR. Based on the adaptation of individual recurrent parents to each partner country, these RLs represent superior germplasm with effective APR to stripe rust that can be further selected and developed for potential release as new varieties with durable stripe rust resistance. The APR in these lines is contributed by the donors ISR 1043.15, Diamondbird, Synthetic wheat line AUS30515, and Kingbird.

Comparative analysis of multi-pathotype testing data and field data from all the countries showed that 17 RLs (**Appendix XIII**) were resistant both at the seedling stage and adult plant growth stages. These 17 RLs presumably carry ASR for stripe rust. They represent five crosses (PBW343 x Kingbird [NAM 3.22], Enkoy x Synthetic AUS30515 [NAM 11.7], Digalu x Synthetic AUS30515 [NAM 14.21], Digalu x ISR 1043.15 [NAM 15.6] and Digalu x Kingbird [NAM 16.20]). Notably, RLs from the crosses of high yielding well adapted wheat cultivars

like PBW 343 and Digalu can be further selected and released as improved high yielding and stripe rust resistant cultivars.

Table 3: Number of NAM population recombinant lines expressing different levels of stripe rust resistance when tested across field environments in Australia, Ethiopia, India, Nepal and Pakistan

1–9 WYR scale	Australia	Ethiopia	India	Nepal	Pakistan
1 (VR)	15	0	38	134	295
2 (R)	805	77	322	0	91
3 (R-MR)	338	5	332	484	81
4 (MR)	152	45	248	215	141
5 (MR-MS)	67	286	286	537	282
6 (MS)	30	293	155	21	207
7 (MS-S)	38	407	51	28	12
8 (S)	6	173	19	18	204
9 (VS)	1	166	0	0	115

1–9 WYR scale (Sandhu *et al.* 2021)

Near isogenic lines (NILs)

Near isogenic genotypes (BC_6F_5) carrying the APR genes *YrCK1*, *YrCK2*, *YrCD*, *Yr36* and *YrH* were generated in the Avocet +*Yr18* background. Additional combinations of Avocet +*Yr18* +*Yr29*, Avocet +*Yr18* +*Yr46*, Avocet +*Yr18* +*Yr29* +*Yr46* and Avocet +*Yr29* +*Yr46* were either already available to the project or were generated. These NILs and combinations of APR genes have been tested under field conditions and in the greenhouse at PBI. All the NIL stocks carrying APR genes *YrCK1*, *YrCK2*, *YrCD*, *Yr36* and *YrH* and combinations were susceptible (infection type “3+”) at the seedling stage (1–2 leaf stage) when tested in the greenhouse, except *Yr36* which expressed resistance in the first leaves. These plants were allowed to grow in the greenhouse and then re-inoculated at the 6–7 leaf stage growth stage. Interestingly, all the APR genes (*YrCK1*, *YrCK2*, *YrCD*, *Yr36* and *YrH*) and combinations expressed resistance at the 6th leaf growth sate, well before flag leaf emergence (**Fig. 29**).

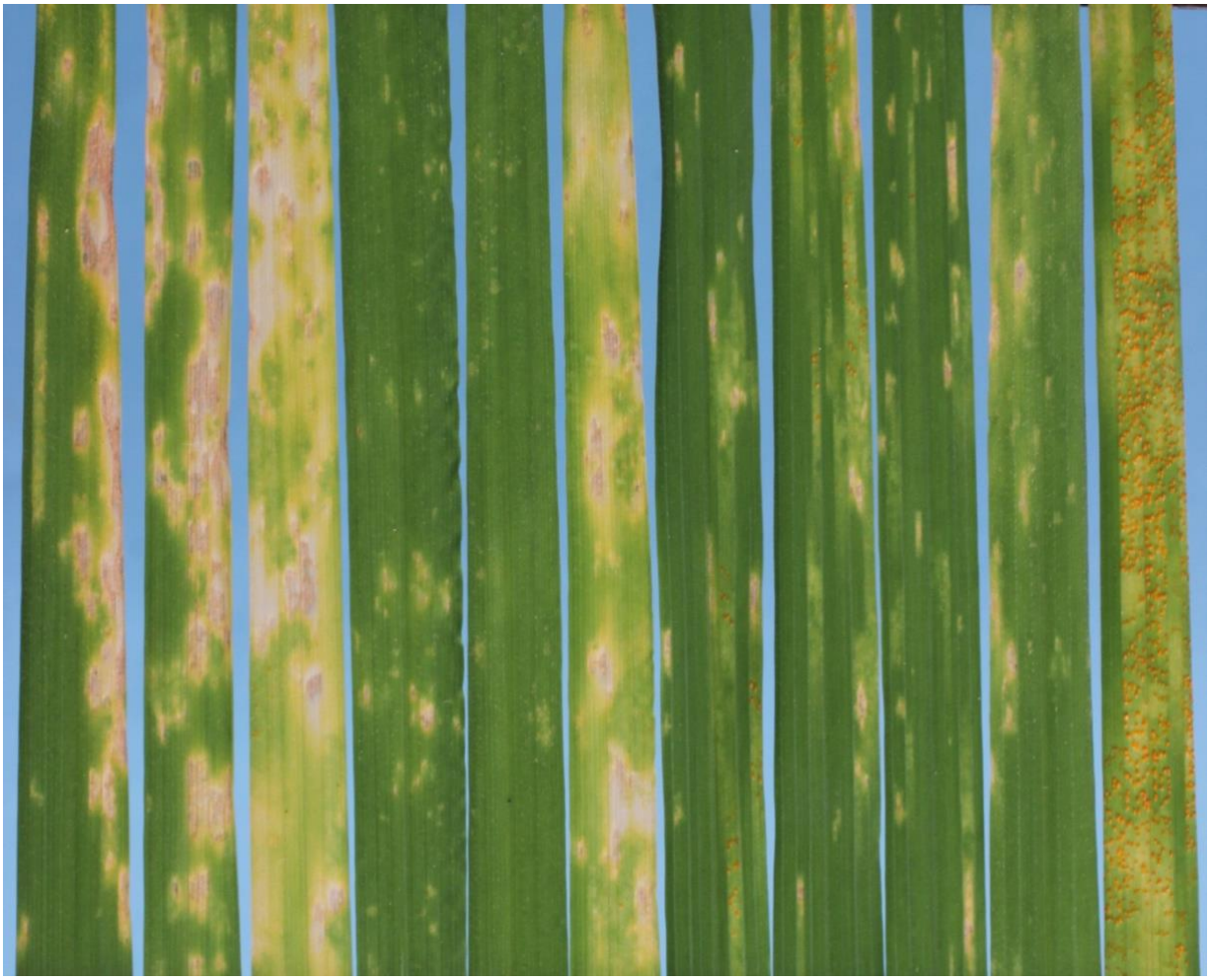

Fig. 29: Responses of the 6th leaf of BC_6F_5 NILs when challenged with WYR under greenhouse conditions, left to right: *YrCK1*, *YrCK2*, *YrYrH*, *YrCD*, *Yr36*, *Yr18*, *Yr29+46*, *Yr18+29+46*, *Yr18+29*, *Yr18+46* and Avocet S

In **Fig. 29**, NILs with APR genes *YrCK1*, *YrCK2*, *YrH*, *YrCD*, *Yr36* in the Avocet +*Yr18* background are resistant with necrosis and no development of rust pustules, as compared to *Yr18* (6th from left) which expressed necrosis but with some rust development as well. Combinations of *Yr29+46* (7th from left), *Yr18+29+46* (8th from left) and *Yr18+29* (9th from left) showed MS response with some rust development and are less effective comparatively. The combination *Yr18+46* (10th from left), with some necrosis but no development of stripe rust, proved to be the best among the NILs at 6th leaf growth stage under greenhouse conditions. When inoculated at the flag leaf growth stage (**Fig. 30**), the level of resistance across the NILS was similar to that seen at the 6th leaf stage. Overall, at the 6th leaf growth stage and later all NILs with APR gene combinations of *Yr18* with *YrCK1*, *YrCK2*, *YrCD*, *Yr36* and *YrH* were significantly more resistant to stripe rust compared to Avocet +*Yr18*.

Fig. 30: Responses of the flag leaves of BC_6F_5 NILs when challenged with WYR under greenhouse conditions, left to right: *YrCK1*, *YrCK2*, *YrYrH*, *YrCD*, *Yr36*, *Yr18*, *Yr29+46*, *Yr18+29+46*, *Yr18+29*, *Yr18+46* and Avocet S

All the NILs BC_6F_5 of APR genes *YrCK1*, *YrCK2*, *YrH*, *YrCD* and *Yr36* in the Avocet +*Yr18* background expressed strong resistance to stripe rust on flag leaves when tested under field conditions (**Fig. 31**). A comparison of NILs with APR genes *CK1*, *CK2*, *YrH*, *YrCD* and *Yr36* in Avocet +*Yr18* background and APR genes *YrCK1*, *YrCK2*, *YrH*, *YrCD* and *Yr36* alone is shown in **Fig. 32.** APR genes *YrCK1*, *YrCK2*, *YrH*, *YrCD* and *Yr36* in the Avocet +*Yr18* background provided better stripe protection in comparison to the APR genes *YrCK1*, *YrCK2*, *YrH*, *YrCD* and *Yr36* alone, but there was no noticeable difference between the level of resistance provided by *Yr36* alone or in combination with *Yr18*.

Among the NILs and APR gene combinations, Avocet +*Yr36* and Avocet +*Yr18* +*Yr46* proved to be highly resistant against stripe rust. According to the 1–9 WYR scale, NILs with APR genes *YrCK1*, *YrCK2*, *YrH*, *YrCD* and *Yr36* in the Avocet +*Yr18* background were scored for stripe rust response on flag leaves under field conditions as 5, 6, 5, 4 and 1, respectively. APR gene *Yr18* and the combinations *Yr29+46*, *Yr18+29+46*, *Yr18+29*, and *Yr18+46* were scored as 7, 7, 5, 6 and 1, respectively.

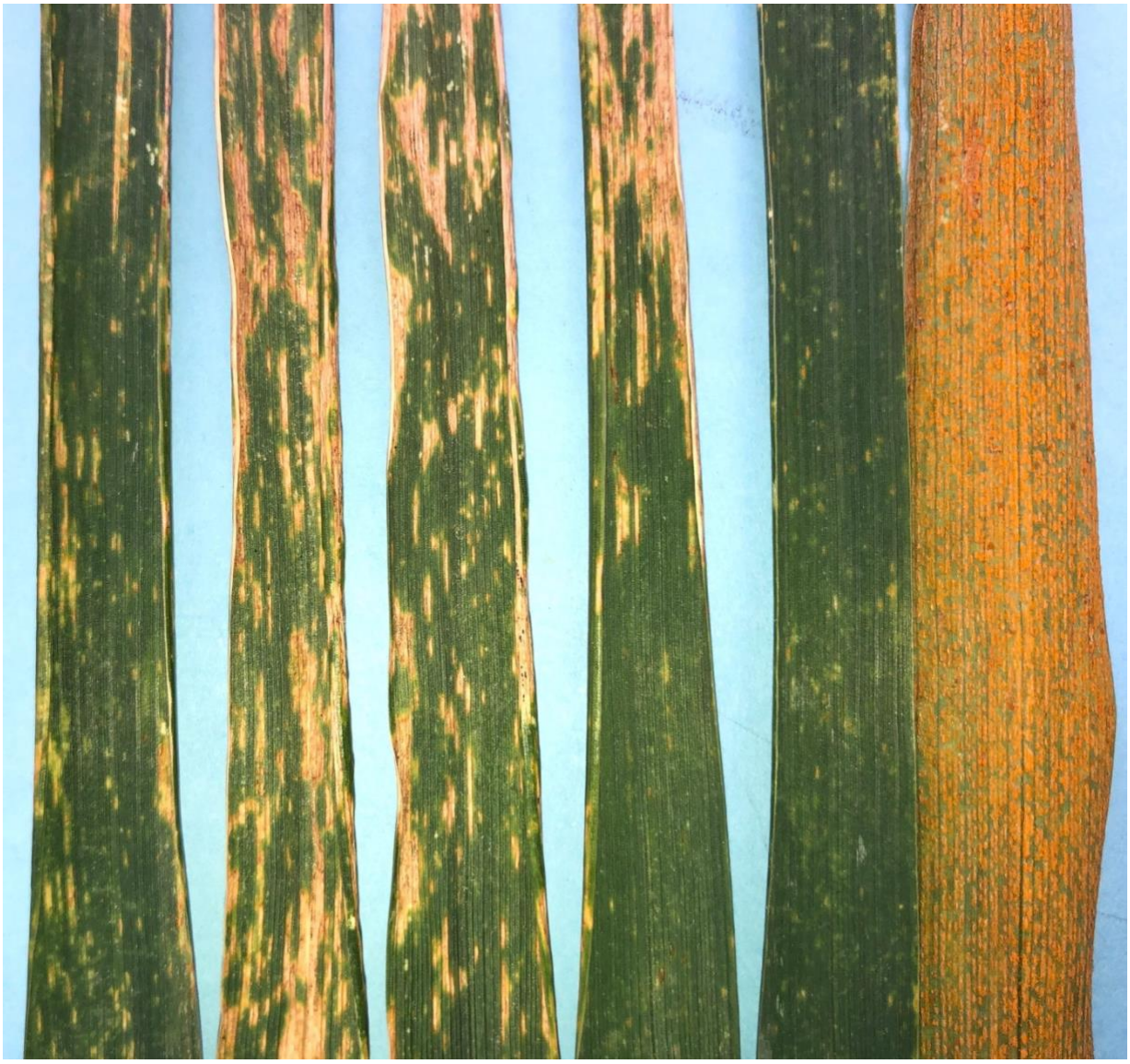

Fig. 31: Responses of the flag leaves of BC_6F_5 NILs when challenged with WYR under field conditions, left to right: *YrCK1*, *YrCK2*, *YrYrH*, *YrCD*, *Yr36*, and Avocet S

The APR gene combination *Yr18+46* was also found to be resistant against WLR under field conditions. Flag leaf and Flag leaf-1 of *Yr18+46* in Avocet S background expressed resistance as compared to susceptible Morocco under field conditions (**Fig. 33**). This was not unexpected given that both *Yr18* and *Yr46* are pleiotropic loci that confer resistance to not only stripe rust, but also leaf rust, stem rust and powdery mildew.

These NIL stocks will allow precise assessments of the performance of the genes singly and in combination under different environments, allowing far better precision in estimating yield loss through replicated field trials. In situations where one or more gene/ gene combinations perform poorly, the lines can then be used in the greenhouse to compare pathogen isolates to determine pathogen virulence for minor resistance genes.

An additional 22 NILs carrying known APR genes for stripe rust resistance are currently under development. Our ultimate aim is to have a complete set of NIL lines with known APR genes present singly and in combinations of 2+ genes so that the genes can be deployed in agriculture with a sound understanding of performance, and monitored post-release.

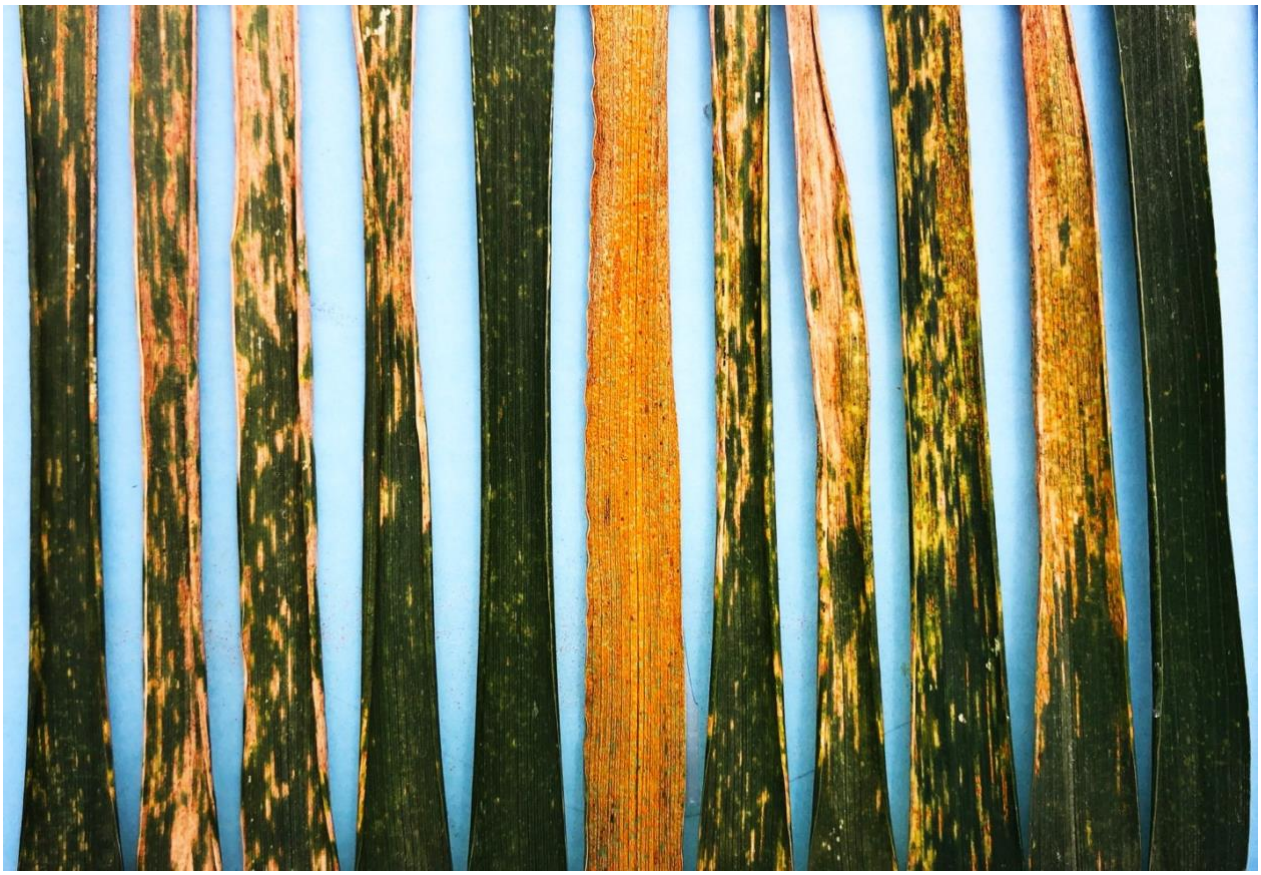

Fig. 32: Responses of the flag leaves of BC_6F_5 NILs when challenged with WYR under field conditions, left to right: *YrCK1+18*, *YrCK2+18*, *YrH+18*, *YrCD+18*, *Yr36+18*, Avocet S, *YrCK1* alone, *YrCK2* alone, *YrH* alone, *YrCD* alone, and *Yr36* alone.

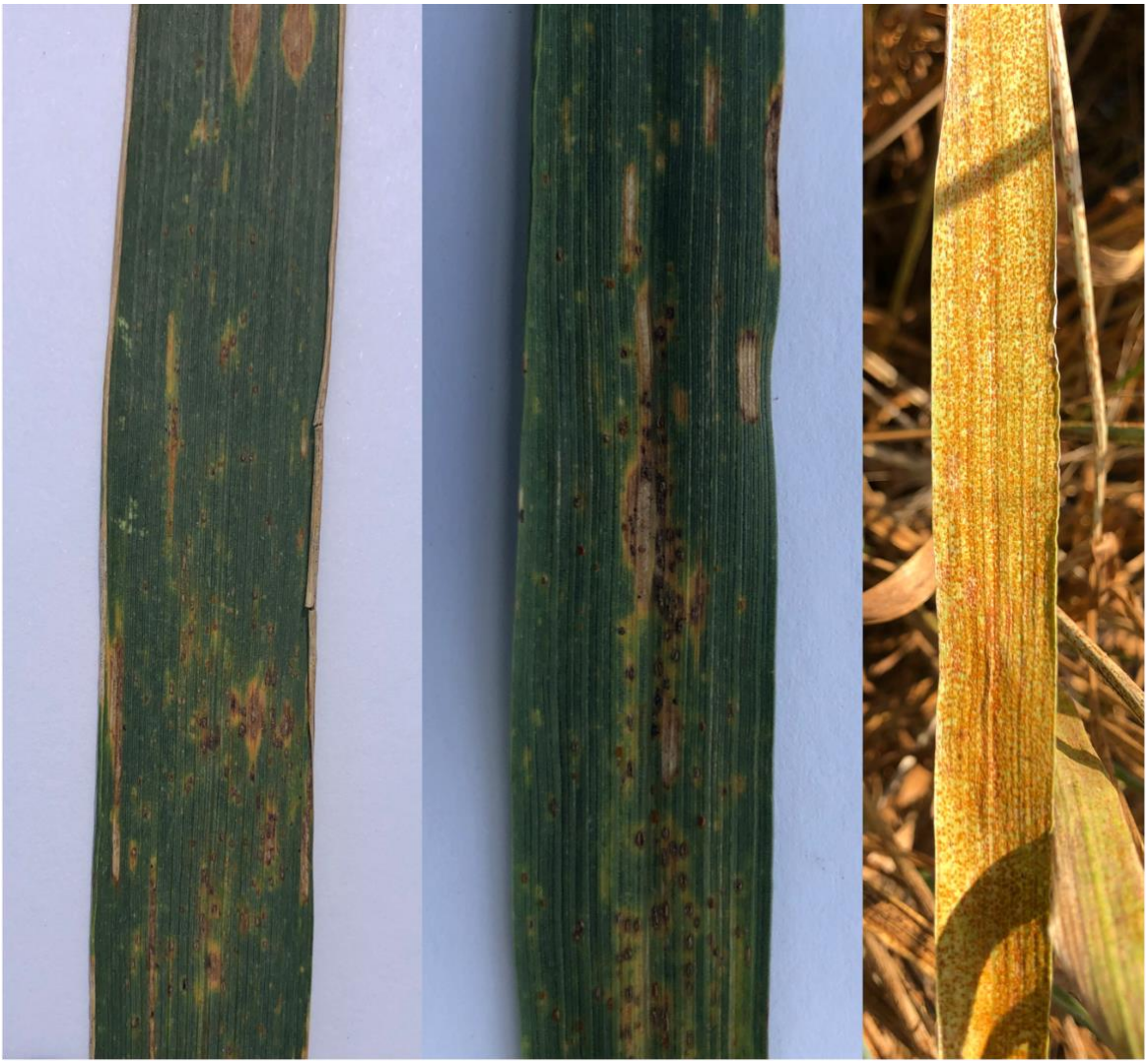

Fig. 33: Responses of the flag leaves of BC_6F_5 NILs when challenged with WLR under field conditions, left to right: *Yr18+46 and* susceptible Morocco

Doubled Haploid (DH) populations

Twelve DH populations were developed to map important high value broadly effective genes conferring adult plant resistance to stripe rust.

Seed of each of 1,812 lines from the 12 DH populations including parents were sent to all partners countries except Ethiopia for field phenotyping. The lines were screened for stripe rust resistance at PBI during the crop season 2021 and during 2021-22 in India, Nepal and Pakistan. The populations segregated for different numbers of genes for WYR resistance when tested under different field environments. Segregation and genetic ratios under the environments of Australia, India, Nepal and Pakistan are described in **Tables 4**, **5**, **6** and **7**, respectively.

At Karnal in India, WYR did not develop well in the current crop season and many DH populations did not segregate for stripe rust response at all (**Table 5**). Due to the early onset of summer, the development of WYR was also sub-optimal in Nepal and Pakistan. In contrast, 2021 was a perfect year for WYR development in Australia in artificially inoculated rust trials at PBI. The number of genes for *Pst* resistance expressed under the environment of Australia, India, Nepal and Pakistan are summarised for comparison in **Table 8**. Although there was some commonality in the number of genes detected in populations that were tested in India and Nepal, significant differences were observed between the number of genes detected between these countries and Australia and Pakistan. These differences could be due to the presence of ASR genes that were effective in one environment and not in another, or other factors. Further work, including mapping the QTL detected, is required to account for these differences. The populations will require field rust screening for at least two more crop seasons to collect data required for the mapping of the unknown gene/s for WYR resistance.

Table 4: Prediction of *Puccinia striiformis* f. sp. *tritici* resistance gene/s in Doubled haploid populations tested under field conditions in Australia

DH Pop ID	Parents R/S	Res. Lines	Susc. Lines	χ2	Ratio R/S	Gene/s
K1	AUS30521/Avs	45	33	1.84	1:1	1
K2	AUS34169/Avs	109	18	0.32	7:1	3
K3	AUS34198/Avs	77	18	3.6	7:1	3
K4	Braewood/Avs	22	11	1.2	3:1	2
K5	Carinya/Avs	64	54	0.84	1:1	1
K6	EGA Stampede/Avs	243	71	0.95	3:1	2
K7	Giles/Avs	43	46	0.1	1:1	1
K8	Monad/Avs	32	9	3.34	7:1	3
K9	Danphe/Avs	59	26	1.41	3:1	2
K10	Kenya Kudu/Avs	176	68	1.07	3:1	2
K11	Kingbird/Avs	197	55	1.35	3:1	2
K12	Munal#1/Avs	158	68	3.12	3:1	2

χ^2 table value at P = 0.05 is 3.84 (1 d.f.)

Table 5: Prediction of *Puccinia striiformis* f. sp. *tritici* resistance gene/s in Doubled haploid populations tested under field conditions in India

DH Pop ID	Parents R/S	Res. Lines	Susc. Lines	χ2	Ratio R/S	Gene/s
K1	AUS30521/Avs	79	0			?
K2	AUS34169/Avs	127	1	6.53	15:1	4
K3	AUS34198/Avs	90	0			?
K4	Braewood/Avs	37	0			?
K5	Carinya/Avs	NA	NA			NA
K6	EGA Stampede/Avs	NA	NA			NA
K7	Giles/Avs	NA	NA			NA
K8	Monad/Avs	53	0			?
K9	Danphe/Avs	82	0			?
K10	Kenya Kudu/Avs	231	0			?
K11	Kingbird/Avs	238	2	4.16		5
K12	Munal#1/Avs	NA	NA			NA

χ^2 table value at P = 0.01 is 6.63 (1 d.f.)

Table 6: Prediction of *Puccinia striiformis* f. sp. *tritici* resistance gene/s in Doubled haploid populations tested under field conditions in Nepal

DH Pop ID	Parents R/S	Res. Lines	Susc. Lines	χ2	Ratio R/S	Gene/s
K1	AUS30521/Avs	70	8	2.13	15:1	4
K2	AUS34169/Avs	124	11	0.83	15:1	4
K3	AUS34198/Avs	85	4	0.55	31:1	5
K4	Braewood/Avs	44	2	0.22	31:1	5
K5	Carinya/Avs	141	5	0.04	31:1	5
K6	EGA Stampede/Avs	301	8	0.29	31:1	5
K7	Giles/Avs	89	0			?
K8	Monad/Avs	51	3	1.05	31:1	5
K9	Danphe/Avs	75	1	0.82	31:1	5
K10	Kenya Kudu/Avs	223	11	1.9	31:1	5
K11	Kingbird/Avs	239	12	2.27	31:1	5
K12	Munal#1/Avs	204	11	2.81	31:1	5

χ^2 table value at P = 0.05 is 3.84 (1 d.f.)

Table 7: Prediction of *Puccinia striiformis* f. sp. *tritici* resistance gene/s in Doubled haploid populations tested under field conditions in Pakistan

DH Pop ID	Parents R/S	Res. Lines	Susc. Lines	χ2	Ratio R/S	Gene/s
K1	AUS30521/Avs	50	11	1.7	7:1	3
K2	AUS34169/Avs	70	58	1.12	1:1	1
K3	AUS34198/Avs	50	41	0.89	1:1	1
K4	Braewood/Avs	29	18	4.43	3:1	2
K5	Carinya/Avs	82	60	3.4	1:1	1
K6	EGA Stampede/Avs	220	79	0.32	3:1	2
K7	Giles/Avs	44	43	0.01	1:1	1
K8	Monad/Avs	43	8	0.47	7:1	3
K9	Danphe/Avs	45	34	1.53	1:1	1
K10	Kenya Kudu/Avs	160	59	0.43	3:1	2
K11	Kingbird/Avs	173	75	3.63	3:1	2
K12	Munal#1/Avs	104	102	0.01	1:1	1

χ^2 table value at P = 0.05 is 3.84 (1 d.f.) and at P = 0.01 is 6.63 (1 d.f.)

Table 8: Number of *Puccinia striiformis* f. sp. *tritici* resistance gene/s in Doubled haploid populations expressed under the field conditions of Australia, India, Nepal and Pakistan

DH Pop ID	Parents R/S	Australia	India	Nepal	Pakistan
K1	AUS30521/Avs	1	?	4	3
K2	AUS34169/Avs	3	4	4	1
K3	AUS34198/Avs	3	?	5	1
K4	Braewood/Avs	2	?	5	2
K5	Carinya/Avs	1	NA	5	1
K6	EGA Stampede/Avs	2	NA	5	2
K7	Giles/Avs	1	NA	?	1
K8	Monad/Avs	3	?	5	3
K9	Danphe/Avs	2	?	5	1
K10	Kenya Kudu/Avs	2	?	5	2
K11	Kingbird/Avs	2	5	5	2
K12	Munal#1/Avs	2	NA	5	1

8 Impacts

We anticipated that initial benefits from the project research outcomes and training would begin to flow by completion, with optimal impact being reached after about 10 years. Overall benefits will be realised principally by a reduction in damage to wheat crops caused by stripe rust, due to the deployment of durable and effective resistance to this disease. This will help to stabilise wheat production, and reduce or eliminate the use of fungicides, resulting in reduced costs of production, safer food for consumers, safer workplaces and a cleaner environment. To give some idea of the value of this approach, it was estimated in 2009 that genetic protection from stripe rust in Australia returns savings of some $431 million annually.

8.1 Scientific impacts – now and in 5 years

The principal underlying research theme of this project was to undertake a critical appraisal of the effectiveness of known and unknown minor gene resistance to stripe rust across different regions. Although minor gene resistance to stripe rust is considered to be durable, there are examples in both the developed and developing world where it has not performed as well as expected once deployed in high yielding cultivars. This is either because the expression of the minor genes underlying the resistance is affected by environment, because the stripe rust pathogen has developed virulence for the minor gene in question, or both. Having the information and resources needed to answer this question is critical as wheat breeding programs around the world are becoming increasingly reliant on minor gene resistance to stripe rust due to the rapid demise of major ASR genes following deployment. The effectiveness and durability of minor gene resistance are also important scientific questions as it has been often assumed that these genes are not vulnerable to pathogen adaptation.

We now have information from the Core Set that will allow us to identify not only combinations of minor genes that provide effective protection against stripe rust under diverse environmental conditions, but also which minor genes do not. Our partners were vital in compiling the Core Set, and in compiling the data set that underpins this work. We have almost finalised the development of a first cohort of near isogenic genotypes that can be used to determine the protective value of single and multiple minor genes, and to understand and monitor pathogen virulence for minor gene resistance. The germplasm and data generated in this project is expected to have high scientific impact as no other project has attempted to address minor gene durability and effectiveness to date.

We also finalised the development of a Nested Association Mapping population, and shown that it includes lines with local adaptation that have very high levels of APR to stripe rust in

our partner countries. This resource will aid in the development of markers linked to important minor genes for resistance to stripe rust. Several genotypes from the NAM population, the Core Set, and the synthetic wheats screened for rust resistance in India, Nepal and Pakistan are of interest to wheat breeders and we expect that these will be included in local wheat breeding programs.

8.2 Capacity impacts – now and in 5 years

Training in Rust Pathology and Genetics

Under this project, scientists from the partner countries who were trained at PBI in "Rust Genetics and Pathology" have already begun to provide support to their local cereal breeding and pathology programs. This was evident from the independent rust scoring conducted by these scientists in Ethiopia, India, Nepal and Pakistan, during the last crop season. Partners have acknowledged that training provided at PBI was a big success, not only for helping to achieve project outcomes but also by contributing to other work being undertaken by the partner institutes. We believe that this comprehensive training has equipped them with the skills and knowledge to allow them to identify, characterise and exploit new sources of rust resistance. The timing of the training was most fortuitous because the trainees were able to contribute to local project activities at a time when Australian scientists were unable to travel due to Covid 19 restrictions. The PBI trained scientists are also providing support for in-country rust race analysis and maintenance of pure rust inoculum, rust screening of germplasm in the greenhouse and marker assisted selections for breeding disease resistant wheat varieties. Six early career scientists from Ethiopia, India and Nepal who spent five months undertaking training in "Rust Pathology and Genetics" at PBI, can play an important role for their local wheat breeding programs, in terms of germplasm selections, rust survey and further providing training to their local students and staff.

Fig. 34: Group of junior scientists from the partner countries trained at PBI during 2019

From L to R: Mr. Prem Magar (Nepal), Dr. Satish Kumar (India), Mr. Dawit Asnake (Ethiopia), Prof. Robert Pak (PBI), Ms. Aline Casassola (Brazil), Dr. Karanjeet Sandhu (PBI), Mr. Shiwarttan Gupt (Nepal), Mr. Tamene Sarbesa (Ethiopia) and Dr. Pramod Prasad (India)

8.3 Community impacts – now and in 5 years

The release of rust resistant wheat cultivars can reduce the risk of crop failures and help in providing a sustained income for smallholder farmers. We firmly believe that in the coming years, the germplasm, knowledge and training provided under this project will have tangible effects in reducing the impact of stripe rust in our partner countries of Ethiopia, India, Nepal and Pakistan.

8.3.1 Economic impacts

Goyal and Manoharachary (2014) considered stripe rust as the most damaging disease of wheat crop on a global scale, and further Beddow *et al.* (2015) estimated that 88% of the world's wheat production is vulnerable to infection and the disease is responsible for the loss of some 5.47 million tonnes of wheat per annum (equivalent to a loss of US$979 million per

year). We anticipate that the germplasm developed under this project will reduce losses and the risk of future losses. Breeding rust resistant wheat cultivars and their post-release management will reduce the impact of WYR in EA and SA, which will contribute to stabilising the incomes of marginal farmers. The economic impacts will include the benefits in terms of reduced risks of WYR epidemics, yield losses avoided, and fungicide costs not incurred. Murray and Brennan (2009) analyses of costs and benefits have shown a very high value of genetic approaches to rust control in Australia, with estimated annual savings due to genetic control and fungicidal control of WYR in Australia at $431 million and $359 million. So, a noticeable economic impact is expected once WYR resistant cultivars are released and promoted in Ethiopia, India, Nepal and Pakistan.

One thing that is often not realised in plant breeding is the cost and effort needed simply to maintain the progress that has been made in yield gains and especially disease resistance in the face of continuing pathogen evolution. Such maintenance breeding has been estimated to absorb at least 50% of the costs of breeding a new variety. We believe our work will underpin these maintenance breeding efforts, the outcomes of which are not increased yields but rather absence of disease.

8.3.2 Social impacts

Stabilizing crops yields by sustainable disease control is a crucial part of poverty alleviation, which has been shown time and again to lead to continued improvements in livelihoods via factors such as greater access to education. Dr Norman Borlaug, architect of the Green Revolution, once said “you cannot build a peaceful world on empty stomachs and human misery”. Based on the resistant germplasm developed under the project, the release of rust resistant wheat cultivars is highly likely to contribute to poverty alleviation by stabilizing wheat yields and reducing volatility in farmer incomes. A sustainable control of this disease and reductions in the costs associated with the WYR control in Ethiopia, India, Nepal and Pakistan, will contribute to increasing the income of farmers which will enable them to improve their living standard. Particularly in Sub-Saharan Africa and South and West Asia, girls continue to suffer severe disadvantage and exclusion in education systems, and while there are several barriers preventing their participation, a significant factor is that families cannot afford to educate all children, with boys being favoured. Farmers with sustained income should be able to spend on education and healthcare of their families, and any improvement in the livelihoods of small holder farmers will make a tangible contribution to rectifying this gender imbalance.

8.3.3 Environmental impacts

The discovery and deployment of stripe rust resistance genes alone or in combination with other durable genes will allow the development of wheat cultivars with high levels of durable rust resistance, which will reduce the use of fungicides. Resistance to the DMI “triazole” fungicides in human Aspergillus diseases is becoming more common in several countries, including India. Triazole fungicides and the selection of resistance to medical triazoles in the opportunistic mould Aspergillus fumigatus. There is strong evidence that triazole resistance in fungi that infect humans is developing in the environment because of these chemicals being used in crop protection Verweij *et al.* (2009). In view of this, limiting or eliminating their use in agriculture would seem highly desirable in protecting human health. It is expected that such reductions will reduce chemical residues and lower the risks posed by these chemicals to the health of humans and other animals.

The project will contribute significantly to the resilience of the wheat industry to potential future incursions of stripe rust in Australia, Ethiopia, India, Nepal and Pakistan. The project benefits will begin to flow in few years, with maximum impact being reached after about 10 years. Benefits will be in terms of reductions in the impact of WYR in wheat production that will lead to yield stability and increased income of the farmers. It was estimated in 2009 that genetic protection from WYR in Australian wheat crops returns some $431 million annually. Reducing or even eliminating the need for fungicide intervention in controlling WYR will also result in safer food for consumers, safer workplaces, and a cleaner environment.

8.4 Communication and dissemination activities

Throughout the project, the Australian team was in touch with the project partners through travel, emails and phone calls. Dr Sandhu always contacted partners from Ethiopia, India, Nepal and Pakistan before the start of wheat crop season, during the season and after the season in each country. Partners were advised right from the beginning including the planning of experiments, seed distribution, sowing of trials, data collection and harvesting. We had regular annual meetings where we discussed the results and planned research activities. In 2018, an annual meeting was held in conjunction with the BGRI workshop, and CIMMYT team was also invited to attend meeting which resulted in good discussion over the research results. During the mid-term review and annual meetings in 2019 at Kulumsa in Ethiopia, a big team involving the different scientists from EIAR was invited in addition to the project personnel. During our visit to the partner countries, we also visited other research institutes and universities to promote our project.

We have published project newsletters and promoted these through mass emails to the scientists and uploading these at the University of Sydney website, on AIK SAATH online platform run by ACIAR office Pakistan and by sending these to the ACIAR media team.

We published manuscript entitled “A pictorial disease assessment scale for assessing wheat stripe rust at adult plant growth stage” in *Australasian Plant Pathology*. Another manuscript on the stripe rust resistance of synthetic wheat genotypes is in progress. We are also preparing a manuscript on the results of the Core Set GWAS study. Ancillary to the project were publications by members of the project team on the origins of all four exotic stripe rust incursions into Australia and the identification of gene targets for improved DNA-based pathogen diagnostics (Ding et al., 2021), mapping resistance to stripe rust and other rust diseases of wheat (Kankwatsa et al. (2017), (2019)), of movement of rust from Africa to Australia (Visser et al. 2019), and the development of a nearly complete haplotype phased reference assembly for the stripe rust pathogen (Schwessinger et al. 2018).

9 Conclusions and recommendations

9.1 Conclusions

Scientists from Australia and the partner countries Ethiopia, India, Nepal and Pakistan worked in collaboration and developed rust resistant material and knowledgebase to mitigate the effects of stripe rust on wheat production in SA and EA. In a nutshell, following conclusions are drawn:

1. Wheat germplasm characterised with triple rust resistance (WYR, WLR and WSR resistance) and seed distributed.
2. Wheat germplasm identified and characterised with stripe rust APR which is effective in all the partner countries.
3. Synthetic wheat lines characterised with WYR resistance, and a diverse set of these genotypes made available to partners.
4. Genotyping revealed stripe rust APR specific QTL which provided WYR resistance under different environments.
5. Well adapted wheat cultivars selected from the partner countries were improved through the back crossing of rust resistance from donors from Australia, and this NAM population allowed partners to select the most promising genotypes.
6. Further significant QTL are expected from the sequencing of NAM population.
7. Near isogenic stocks carrying stripe rust APR genes, and other combinations of known stripe rust APR genes developed for monitoring the *Pst* virulence in rust survey.
8. Doubled Haploid populations will allow the mapping of unknown genes characterised for *Pst* resistance.
9. Training of junior scientists helped in building partners capacity in Rust Pathology and Genetics.
10. Research results were promoted at international conferences and at ACRCP meetings in Australia. Advised private sector wheat breeding company to promote the rust resistant wheat germplasm.
11. Project allowed the development of professional relationships between the scientists from Australia, Ethiopia, India, Nepal and Pakistan.

9.2 Recommendations

The project has legacies of well characterised and improved wheat germplasm, data on rust resistance, and important NIL stocks carrying high value minor genes for resistance to stripe rust. We would like to endorse the use of these resources and advocate the following recommendations:

1. Project partners should make further selections from 147 Core Set genotypes that expressed triple rust resistance under Australian condition.
2. Use of donors from 50 Core Set genotypes [(Australia (7), Ethiopia (8), India (17), Nepal (10) and Pakistan (8))] characterised with stripe rust APR, for breeding wheat cultivars with durable rust resistance.
3. Validation of the 20 significant QTL detected from GWAS of Core Set and provide markers for marker assisted selections.
4. Most of the Core Set genotypes were resistant to YLS when tested in Australia, and these resources should be considered to develop multi disease resistant wheat cultivars.
5. Synthetic wheat genotypes characterised with high levels (VR–RMR) of resistance to stripe rust can be used as donors for rust resistance, increasing genetic diversity in the resistances deployed.
6. Wheat breeders should assess the value to their programs of the 127 wheat genotypes out of 300 selected, which expressed resistance against all three wheat rust diseases.
7. Partners are advised to make selections from 127 NAM population RLs which are considered to carry stripe rust APR only in Australia, and further they can narrow their selections by considering 49 out of these 127 RLs that remained resistant under field conditions of Ethiopia, India, Nepal and Pakistan as well.
8. Make selections from 17 NAM population RLs carrying ASR and combine these genes with APR to achieve durable rust resistance.
9. NAM population RLs from the crosses of high yielding well adapted wheat cultivars like PBW 343 and Digalu [PBW343 x Kingbird (NAM 3.22), Digalu x Synthetic AUS30515 (NAM 14.21), Digalu x ISR 1043.15 (NAM 15.6) and Digalu x Kingbird (NAM 16.20)], can be further selected and released directly as improved high yielding and rust resistant cultivars.
10. Include Near isogenic stocks carrying genes *YrCK1*, *YrCK2*, *YrCD*, *Yr36* and *YrH*, and APR genes combinations like Avocet + *Yr18* +*Yr29*, Avocet + *Yr18* +*Yr46*, Avocet + *Yr18* +*Yr29* +*Yr46* and Avocet + *Yr29* +*Yr46* in rust surveys to monitor *Pst* virulence for stripe rust APR genes.

11. Develop single APR genes carrying NILs by crossing *YrCK1*, *YrCK2*, *YrCD*, *Yr36* and *YrH* with Avocet S.
12. Develop more combinations of APR genes by crossing *YrCK1*, *YrCK2*, *YrCD*, *Yr36* and *YrH* with Avocet + *Yr18* +*Yr29*, Avocet + *Yr18* +*Yr46*, Avocet + *Yr18* +*Yr29* +*Yr46* and Avocet + *Yr29* +*Yr46* and test these APR combinations across the environments.
13. NILs carrying APR genes can be used to test the best combinations for additive for gene pyramiding.
14. Test DH populations at least for one more crop season.
15. Genotyping by Sequencing of DH populations is recommended for the mapping of unknown genes of stripe rust resistance.
16. Collaboration with private seed companies is recommended to speed up the breeding of rust resistant cultivars using germplasm developed and or characterised in the project.

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ACIAR Project: CIM/2014/081Fact Sheet (https://www.aciar.gov.au/project/cim-2014-081)

11 Appendixes (Attached)

Appendix 1: Core Set including 50 cultivars each from Australia, Ethiopia, India, Nepal and Pakistan

Appendix II: Details of additional germplasm selected including 300 wheat lines

Appendix III: Pedigree details of 94 synthetic wheat lines selected and characterised for rust resistance

Appendix IV: Detail of *Puccinia striiformis* f. sp. *tritici* pathotypes used in the study

Appendix V: Detail of control differentials used in the greenhouse rust testing for gene postulations

Appendix VI: Scale used in the greenhouse for scoring rust infection types at seedling growth stage

Appendix VII Oligonucleotide primers and their PCR profiles used for the amplification of markers linked with *Yr18*, *Yr36* (*WKS*) and *Yr46* genes

Appendix VIII: Details of parents of doubled haploid populations developed at PBI

Appendix IX: Details of 12 doubled haploid populations (1788 Lines) developed at PBI

Appendix X: Out of 250 total 147 Core Set lines characterised with triple rust resistance in Australia

Appendix XI: NAM recombinants (127) found susceptible against the Australian Pathotypes of *Puccinia striiformis* f. sp. *tritici* used at seedling growth stage and all remained resistant at adult plant growth stage in Australia, except few in Ethiopia, India, Nepal and Pakistan

Appendix XII: NAM recombinants (49) found susceptible against the Australian Pathotypes of *Puccinia striiformis* f. sp. *tritici* used at seedling growth stage and all remained resistant at adult plant growth stage across the environments of Australia, Ethiopia, India, Nepal and Pakistan

Appendix XIII: NAM recombinants (17) found resistant against all the Australian Pathotypes of *Puccinia striiformis* f. sp. *tritici* used at seedling stage and resistant at adult plant growth stage across the environments of Australia, Ethiopia, India, Nepal and Pakistan