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ORIGINAL RESEARCH ARTICLE

Identification of synthetic wheat lines with broadly effective stripe rust resistance

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Abstract

Wheat stripe/yellow rust (WYR), caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is a major constraint in global wheat production. A set of 766 hexaploid synthetic wheat lines, including primary crosses of *Triticum turgidum* x *Aegilops tauschii* and their derivatives, were screened in artificially rust inoculated field nurseries for three seasons. From this set, a core set of 94 non-lodging lines with unique pedigrees and resistance to *Pst* that was consistent across years was established. The core set was tested for adult plant field response under field conditions for three seasons in Australia and at least one crop season in Ethiopia, India, Kenya, Nepal and Pakistan. It was also challenged with an array of well-defined *Pst* pathotypes at seedling growth stages in the greenhouse, and genotyped with molecular markers linked to the adult plant resistance (APR) genes *Yr18*, *Yr36* and *Yr46*. Combined analysis of field rust responses, multi-pathotype seedling phenotyping and marker genotyping resolved seven classes of *Pst* resistance: uncatalogued (new) APR (UAPR, 11%), uncatalogued seedling resistance (USR, 46%), known seedling resistance (KSR, 5%), KSR + USR (2%), *Yr18* + UAPR (4%), *Yr18* + USR (29%) and *Yr18* + KSR (3%). A majority of the lines carrying UAPR and USR either singly or in combination showed high levels of field resistance across all field sites and years of testing, demonstrating that these lines represent a valuable resource for breeding wheat for resistance to *Pst*.

Keywords Wheat · Stripe rust · Yellow rust · All stage resistance · Adult plant resistance · Synthetic hexaploid · *Puccinia striiformis* f. sp. *tritici*

Introduction

Bread wheat (*Triticum aestivum*) and durum wheat (*T. durum*) are grown worldwide and provide important sources of food and nutrition for humans, providing about 19–20% of the total global dietary calories and 21% of protein (Braun et al. 2010; Tesfaye 2021). About 2.5 billion people eat wheat and its cultivation covers more than 218 million hectares of farmland worldwide under a wide range of climatic conditions (Listman and Ordóñez 2019). World wheat production in 2020 was estimated at 761.7 million tonnes, almost comparable to the world wheat production in 2019 (FAO 2020). World population is expected to reach 9.8 billion in the 2050s, approximately 2.2 billion more than in 2018 (United Nations 2017). To meet projected growing demand, wheat production must increase by at least 60% by 2050 (Wani et al. 2021). As per the FAO's 2020 global report on crop prospects and global food supply, Pakistan

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was projected to import 1.7 million tonnes of wheat in 2020/21 due to tight domestic availability, despite it being traditionally a wheat exporting country. Ethiopia, India and Nepal were predicted to import 1940, 718.5 and 1260 thousand tonnes of cereals, respectively, during 2019/2020 or 2020 (FAO 2020).

The wheat stripe/yellow rust (WYR) causing pathogen *Puccinia striiformis* f. sp. *tritici* (*Pst*) is one of the major biotic limitations to global wheat production and has the potential to cause major yield losses wherever wheat is grown. Epidemics of WYR have resulted in yield losses as high as 100% (Wellings 2011; Chen 2020). Beddow et al. (2015) estimated that 88% of the world's wheat crops were vulnerable to *Pst* infection, with annual loss estimates of 5.47 million tonnes of wheat worth US$979 million.

WYR can be successfully managed within and between cropping cycles by means of genetic resistance, reducing or even eliminating the need for fungicide intervention and resulting in increased productivity and a cleaner environment. To give some idea of the value of this approach, it was estimated in 2009 that genetic protection from WYR in Australia returns some $431 million annually (Murray and Brennan 2009). An example of the benefit of genetic resistance in controlling WYR in Australia is demonstrated by the much lower impact of *Pst* pathotype 198E16A-J+T+17+, a member of the PstS13 genetic lineage that caused widespread yield losses in Argentina and Europe (Park et al. 2020; Ding et al. 2021).

So far, 89 alleles at 83 loci governing resistance to *Pst* have been catalogued in wheat (KOMUGI 2023). Of these, 62 are regarded as major genes, conferring all stage resistance (ASR), and 27 as minor genes that confer adult plant resistance (APR). Of these, five ASR ((*Yr5*, *Yr7*, *YrSP*) (Marchal et al. 2018)), ((*Yr10* (Liu et al. 2014)) and *Yr15* (Klymiuk et al. 2018)), two APR ((*Yr18* (Krattinger et al. 2009) and *Yr46* (Moore et al. 2015)) and one HTP (*Yr36* (Fu et al. 2009)) genes have been cloned so far.

Most of the ASR genes conferring resistance to *Pst* that have been catalogued so far have proven race specific (Chen 2005). Virulence for most of the commercially harnessed ASR genes (*Yr1*, *Yr2*, *Yr3*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr19*, *Yr20*, *Yr21*, *Yr22*, *Yr23*, *Yr24*, *Yr25*, *Yr26*, *Yr27*, *Yr28*, *Yr31*, *Yr32*, *Yr33*, *Yr35*, *Yr43*, *Yr44*, *Yr76*) has been reported from at least one or more wheat producing country (Calonnec and Johnson 1998; Chen 2005; Chen et al. 2010; Holtz et al. 2013; Kumar et al. 1988; Line and Qayoum 1992; Liu et al. 2016; McIntosh et al. 1995; Park et al. 2020; Sharma-Poudyal et al. 2013; Stubbs 1985; Wan and Chen 2012, 2014; Wan et al. 2004, 2016, 2017; Wellings and Burdon 1992; Wellings et al. 2009; Yang et al. 2013). However, virulence has been rarely recorded for *Yr5* in Australia, India, Tajikistan and Turkey (Wellings et al. 2009) and for *Yr10* in Chile, China, Ethiopia, Kenya, Hungary, Pakistan and Uzbekistan (Sharma-Poudyal et al. 2013; Wan et al. 2017) and Australia. Virulence for ASR gene *Yr15* has been reported only from Afghanistan (Van Silfhout 1989).

In contrast to ASR genes, APR genes have proven to have a greater tendency to be non-race specific and durable, however they can be less effective at protecting against yield loss under high disease pressure (Bariana and McIntosh 1995; McIntosh et al. 1995; Chen and Line 1995; Johnson 1988; Chen 2013). High levels of durable rust resistance can be achieved by incorporating different minor genes. This strategy of combining APR genes with additive effect has been practiced successfully in Australia, to breed rust resistant cultivars (Singh et al. 2001).

The ability of *Pst* to evolve and overcome resistance has resulted in many catalogued ASR genes being rendered ineffective. Although APR genes have proven to be more durable, fewer of these are available to wheat breeders. In view of this, we conducted detailed tests of a large set of synthetic wheat lines (produced by crossing *Aegilops tauschii* Coss. (=*Ae. squarrosa*) with tetraploid wheat), which were previously reported to carry high and diverse levels of stripe rust resistance (Kishii et al. 2019).

Materials and methods

Germplasm

A set of 766 hexaploid synthetic wheat lines including primary crosses of *T. turgidum* x *Ae. tauschii* Coss. (=*Ae. squarrosa* L.) and their derivatives, developed at CIMMYT (Mujeeb-Kazi et al. 1996). were sourced from the "Australian Grains Genebank". All lines were screened for rust response in artificially rust inoculated field nurseries for three consecutive seasons at the Plant Breeding Institute (PBI) Cobbitty. Of these, 591 lines were consistently resistant to *Pst*, from which a core set of 94 non-lodging lines with unique pedigrees was established (Table 1). The control genotypes AvocetS, and two lines near isogenic to AvocetS (NILs) carrying the APR genes *Yr18* (AvocetS + *Yr18*) or *Yr29* (AvocetS + *Yr29*) were sourced from the University of Sydney PBI germplasm collection. The 94 core set lines were increased by selecting a single representative plant from each, with the seed thus derived used for testing across environments, in greenhouse multi-pathotype testing, and for DNA used in genotyping for markers linked with the genes *Yr18*, *Yr36* and *Yr46*.

Pathogen material

For seedling tests, nine Australian pathotypes of *Pst* (104E137A+, 110E143A+, 134E16A+, 134E16A+J+Yr27+, 134E16A+17+27+, 134E16A+J+T+,

Table 1 Core set of Synthetics (n = 94) tested and their pedigree detail

PBI#	AUS No.	Pedigree
Syn_1	AUS30265	FALCIN/AE. SQUARROSA (389)
Syn_2	AUS30266	ARLIN/AE. SQUARROSA (410)
Syn_3	AUS30268	GREEN/AE. SQUARROSA (458)
Syn_4	AUS30269	LARU/AE. SQUARROSA (507)
Syn_5	AUS30273	GAN/AE. SQUARROSA (182)
Syn_6	AUS30275	DOY1/AE. SQUARROSA (255)
Syn_7	AUS30279	SCA/AE. SQUARROSA (272)
Syn_8	AUS30282	DOY1/AE. SQUARROSA (334)
Syn_9	AUS30283	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE. SQUARROSA (334)
Syn_10	AUS30288	CROC_1/AE. SQUARROSA (466)
Syn_11	AUS30307	CROC_1/AE. SQUARROSA (205)//MILAN/KAUZ
Syn_12	AUS30311	ALTAR 84/AE. SQUARROSA (211)//BAV92
Syn_13	AUS30312	CASKOR/3/CROC_1/AE. SQUARROSA (224)//OPATA
Syn_14	AUS30320	CROC_1/AE. SQUARROSA (224)//OPATA/3/PASTOR
Syn_15	AUS30332	PJN/BOW//OPATA*2/3/CROC_1/AE. SQUARROSA (224)//OPATA
Syn_16	AUS30334	URES/BBL//KAUZ/3/KAUZ/4/CHEN/AE. SQUARROSA (TAUS)//BCN/5/URES/JUN//KAUZ
Syn_17	AUS30336	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE. SQUARROSA (208)/5/HAHN/2*WEAVER
Syn_18	AUS30337	CNDO/R143//ENTE/MEXI_2/3/AE. SQUARROSA (TAUS)/4/WEAVER/5/WEAVER
Syn_19	AUS30341	CHIL/2*STAR/5/CNDO/R143//ENTE/MEXI_2/3/AE. SQUARROSA (TAUS)/4/WEAVER
Syn_20	AUS30343	D67.2/P66.270//AE. SQUARROSA (217)/3/IRENA
Syn_21	AUS30344	CROC_1/AE. SQUARROSA (224)//OPATA/3/ATTILA
Syn_22	AUS30353	CNDO/R143//ENTE/MEXI_2/3/AE. SQUARROSA (TAUS)/4/WEAVER/5/BORL95
Syn_23	AUS30357	MILAN/KAUZ/5/CNDO/R143//ENTE/MEXI_2/3/AE. SQUARROSA (TAUS)/4/WEAVER/6/TOB/ERA//TOB/CNO67/3/PLO/4/VEE#5/5/KAUZ
Syn_24	AUS30360	PASTOR/3/ALTAR 84/AE. SQUARROSA (TAUS)//OPATA
Syn_25	AUS30398	TOB/ERA//TOB/CNO67/3/PLO/4/VEE#5/5/KAUZ/6/CNDO/R143//ENTE/MEXI_2/3/AE. SQUARROSA (TAUS)/4/WEAVER/7/URES/JUN//KAUZ
Syn_26	AUS30500	CROC_1/AE. SQUARROSA (210)//2*EXCALIBUR
Syn_27	AUS30503	ALTAR 84/AE. SQUARROSA (211)//2*JANZ
Syn_28	AUS30505	CROC_1/AE. SQUARROSA (224)//2*KULIN
Syn_29	AUS30508	YAV79//DACK/RABI/3/SNIPE/4/AE. SQUARROSA (460)/5/2*EXCALIBUR
Syn_30	AUS30509	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE. SQUARROSA (878)/6/2*M5660
Syn_31	AUS30511	CETA/AE. SQUARROSA (174)//2*FRAME
Syn_32	AUS30515	SLVS/6/FILIN/IRENA/5/CNDO/R143//ENTE/MEXI_2/3/AE. SQUARROSA (TAUS)/4/WEAVER
Syn_33	AUS30521	CROC_1/AE. SQUARROSA (224)//OPATA/3/RAC655
Syn_34	AUS30525	QT6581/4/PASTOR//SITE/MO/3/CHEN/AE. SQUARROSA (TAUS)//BCN
Syn_35	AUS30538	GOROKE/6/FILIN/IRENA/5/CNDO/R143//ENTE/MEXI_2/3/AE. SQUARROSA (TAUS)/4/WEAVER
Syn_36	AUS30542	SLVS/3/CROC_1/AE. SQUARROSA (224)//OPATA
Syn_37	AUS30547	CETA/AE. SQUARROSA (327)//2*CUNNINGHAM
Syn_38	AUS30549	CETA/AE. SQUARROSA (327)//2*JANZ
Syn_39	AUS30550	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE. SQUARROSA (629)/6/2*FRAME
Syn_40	AUS30552	CETA/AE. SQUARROSA (1024)//2*SLVS
Syn_41	AUS30553	DOY1/AE. SQUARROSA (447)//2*CUNNINGHAM
Syn_42	AUS30556	CROC_1/AE. SQUARROSA (205)//BORL95/3/2*KENNEDY
Syn_43	AUS30560	PGO//CROC_1/AE. SQUARROSA (224)/3/2*BORL95/4/2*CO4417.001
Syn_44	AUS30562	CROC_1/AE. SQUARROSA (224)//2*OPATA/3/2*RAC655
Syn_45	AUS30575	DVERD_2/AE. SQUARROSA (214)//2*BCN/3/2*RAC710
Syn_46	AUS30577	CROC_1/AE. SQUARROSA (397)//2*TRIDENT
Syn_47	AUS30581	ACO89/AE. SQUARROSA (309)//RAC710
Syn_48	AUS30582	CROC_1/AE. SQUARROSA (205)//KAUZ/3/GOROKE

Table 1 (continued)

PBI#	AUS No.	Pedigree
Syn_49	AUS30584	CROC_1/AE. SQUARROSA (205)//BORL95/3/KENNEDY
Syn_50	AUS30594	CROC_1/AE. SQUARROSA (224)//2*OPATA/3/RAC655
Syn_51	AUS30596	D67.2/P66.270//AE. SQUARROSA (320)/3/CUNNINGHAM
Syn_52	AUS30611	CNDO/R143//ENTE/MEXI_2/3/AE. SQUARROSA (TAUS)/4/WEAVER/5/2*JANZ
Syn_53	AUS30613	CNDO/R143//ENTE/MEXI_2/3/AE. SQUARROSA (TAUS)/4/WEAVER/5/2*FRAME
Syn_54	AUS30615	SRN/AE. SQUARROSA (358)/4/URES//BUC/PVN/3/KAUZ
Syn_55	AUS30617	K134(60)/VEE//BOW/PVN/3/CROC_1/AE. SQUARROSA (224)//OPATA
Syn_56	AUS30618	OPATA//CROC_1/AE. SQUARROSA (879)/3/URES/JUN//KAUZ
Syn_57	AUS30619	ALTAR 84/AE. SQUARROSA (221)//PASTOR/3/PASTOR
Syn_58	AUS30620	ALTAR 84/AE. SQUARROSA (224)//2*YACO/3/BABAX
Syn_59	AUS30622	CROC_1/AE. SQUARROSA (213)//PGO/3/BABAX
Syn_60	AUS30623	SRN/AE. SQUARROSA (358)/4/URES//BUC/PVN/3/KAUZ/5/BABAX
Syn_61	AUS30624	ALTAR 84/AE. SQUARROSA (TAUS)//OCI/3/VEE/MJI//2*TUI
Syn_62	AUS30626	GAN/AE. SQUARROSA (257)
Syn_63	AUS30632	SRN/AE. SQUARROSA (358)
Syn_64	AUS30633	SCOOP_1/AE. SQUARROSA (358)
Syn_65	AUS30635	GAN/AE. SQUARROSA (408)
Syn_66	AUS30636	STY-US/CELTA//PALS/3/SRN_5/4/AE. SQUARROSA (431)
Syn_67	AUS30658	GAN/AE. SQUARROSA (897)
Syn_68	AUS30664	GARZA/BOY//AE. SQUARROSA (281)
Syn_69	AUS33376	ALTAR 84/AE. SQUARROSA (333)
Syn_70	AUS33381	RASCON/AE. SQUARROSA (367)
Syn_71	AUS33383	DOY1/AE. SQUARROSA (415)
Syn_72	AUS33386	GARZA/BOY//AE. SQUARROSA (433)
Syn_73	AUS33388	DOY1/AE. SQUARROSA (458)
Syn_74	AUS33391	ALTAR 84/AE. SQUARROSA (507)
Syn_75	AUS33394	GARZA/BOY//AE. SQUARROSA (520)
Syn_76	AUS33395	DOY1/AE. SQUARROSA (532)
Syn_77	AUS33398	CETA/AE. SQUARROSA (170)
Syn_78	AUS33401	CROC_1/AE. SQUARROSA (231)
Syn_79	AUS33402	CETA/AE. SQUARROSA (256)
Syn_80	AUS33403	DOY1/AE. SQUARROSA (256)
Syn_81	AUS33406	SKARV_2/AE. SQUARROSA (304)
Syn_82	AUS33408	DOY1/AE. SQUARROSA (322)
Syn_83	AUS33424	CETA/AE. SQUARROSA (1027)
Syn_84	AUS34091	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE. SQUARROSA (629)/6/2*CARNAMAH
Syn_85	AUS34097	CETA/AE. SQUARROSA (1024)//2*M5660
Syn_86	AUS34100	ALTAR 84/AE. SQUARROSA (502)//2*CARNAMAH
Syn_87	AUS34116	DVERD_2/AE. SQUARROSA (1031)//KRICHAUFF/3/KRICHAUFF
Syn_88	AUS34119	CETA/AE. SQUARROSA (1031)//JANZ/3/SUNVALE
Syn_89	AUS34123	CETA/AE. SQUARROSA (1031)//JANZ/3/PELSART
Syn_90	AUS34142	DVERD_2/AE. SQUARROSA (1031)//SUNECA/3/SUNCO
Syn_91	AUS34148	CROC_1/AE. SQUARROSA (210)//EXCALIBUR/3/RAC-702
Syn_92	AUS34169	ALTAR 84/AE. SQUARROSA (211)//JANZ/3/WESTONIA
Syn_93	AUS34198	CROC_1/AE. SQUARROSA (224)//KULIN/3/WESTONIA
Syn_94	AUS34219	YAV79//DACK/RABI/3/SNIPE/4/AE. SQUARROSA (460)/5/EXCALIBUR/6/SUNCO

AE. AEGILOPS

150E16A+, 239E237A-17+33+ and 198E16A-J+T+17+) were used (Table 2). Field nurseries were inoculated with a mixture of two or more of these pathotypes (Table 2).

Greenhouse rust screening

For greenhouse tests, all lines along with differentials (Table 3) were planted in pots filled with a mixture of fine bark and coarse sand and fertilised using "Aquasol®" (100 gm per 10 L of water per 200 pots) prior to sowing. Seedlings of differentials and synthetic wheat lines were raised in 9 cm diameter pots by sowing four clumps (test lines) of each genotype at 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 L of water per 200 pots). Seedlings at the 1.5–2 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. Following inoculation, plants were transferred to an incubation cabinet fitted with an ultrasonic humidifier that maintained greater than 95% relative humidity inside a dark room maintained at 10 °C darkness. After 24 h, plants were moved to a naturally lit microclimate room maintained at 18 ± 2 °C.

Field rust screening

All lines were field tested in Australia (Karalee; 2016 and Horse Unit; 2017 to 2020), Ethiopia (Kulumsa; 2020), India (Karnal; 2020), Kenya (Njoro; 2019 to 2020), Nepal (Khumaltar; 2020) and Pakistan (Islamabad and Nowshehra; 2020). One-meter row plots were sown during June (Australia), May (Ethiopia and Kenya) and in November (India, Nepal and Pakistan). A row of susceptible spreader (mixture of stripe rust-susceptible local genotypes) was sown after every fifth test line row to facilitate 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 lines 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 also established by placing susceptible, greenhouse inoculated Morocco seedlings raised in 9 cm diameter pots within the plots. In all other countries, epidemics of stripe rust were dependent on natural pathogen inoculum. 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 documented in Fig. 1 and Table 4.

Molecular analysis

DNA extraction

Genomic DNA was extracted from leaf tissue using a CTAB (cetyltrimethylammonium bromide) protocol (Doyle and Doyle 1990). A 15 to 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 h above silica beads to dry the leaf tissue. 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

Table 2 Detail of *Puccinia striiformis* f. sp. *tritici* pathotypes used in this study

Pst Pathotype	PBI culture number	Virulence for *Yr* gene/s
104E137A+	414	*Yr2, Yr3, Yr4, Yr25, YrA*
110E143A+	444	*Yr2, Yr3, Yr4, Yr6, Yr7, Yr25, YrA,*
134E16A+	572	*Yr2, Yr6, Yr7, Yr8, Yr9, Yr25, YrA*
150E16A+	598	*Yr2, Yr6, Yr7, Yr8, Yr9, Yr10, YrA*
134E16A+J+Yr27+	607	*Yr2, Yr6, Yr7, Yr8, Yr9, Yr25, Yr27, YrA, YrJ*
134E16A+J+T+	615	*Yr2, Yr6, Yr7, Yr8, Yr9, Yr25, YrA, YrJ, YrT*
134E16A+Yr17+Yr27+	617[a]	*Yr2, Yr6, Yr7, Yr8, Yr9, Yr17, Yr25, Yr27, YrA*
239E237A-17+33+	674[a]	*Yr1, Yr2, Yr3, Yr4, Yr6, Yr7, Yr9, Yr17, Yr25, Yr32, Yr33*
198E16A+J+T+17+	687[a]	*Yr2, Yr4, Yr6, Yr7, Yr8, Yr9, Yr17, YrA, YrJ, YrT, YrSU* (Suwon 92/Omar)

[a]Predominant field pathotypes

Table 3 Greenhouse multi-pathotype testing data from the Australian pathotypes of *Puccinia striiformis* f. sp. *tritici* used in the study

Line ID	AUS No.	Pty1	Pty2	Pty3	Pty4	Pty5	Pty6	Pty7	Pty8	Pty9
Syn_1	AUS30265	0;	0;C	;N	;1CN+	0;	;C	;C	2=N	3+
Syn_2	AUS30266	0	0;C	0;	0	0;	;C	0	;N	3CN
Syn_3	AUS30268	0;	0;C	2+C	33+	3+	3+	3+	2=C	33+CN
Syn_4	AUS30269	3+	22+CN	3+	3+	3+	3+	3+	3+	3N
Syn_5	AUS30273	0	;C	;C	;CN	;C	;C	;	;C	
Syn_6	AUS30275	0	0;CN	2++	;CN	3+	2+C	0	3C	3N
Syn_7	AUS30279	0	;C	3+	3+	33+	33+	3C	3C	3+
Syn_8	AUS30282	0	;C	0;	;CN	0	0;	0;	;N	3CN
Syn_9	AUS30283	0	0	0	;CN	0;C	;C	0	0;C	3C
Syn_10	AUS30288	3+	3+	3+	3+	3+	3+	3+	3+	3+
Syn_11	AUS30307	0	0;CN	3+	33+C	3+	;N	;C	23+C	2-CN
Syn_12	AUS30311	0	0;C	3+	3-C	3+	;N	3+	3+	33+C
Syn_13	AUS30312	0	0;C	;C	;N	2C	;N	0;	2-N	;N
Syn_14	AUS30320	0;	0;C	0;C	0;	3+	2+CN	;C	;1+CN	3-CN
Syn_15	AUS30332	3+	3+	3+	3+	3+	3+	3+	3+	3+
Syn_16	AUS30334	0	0;CN	3+	3+	3+	3+	3+	3+	3+
Syn_17	AUS30336	0;N	;CN	;CN	;CN	0;C	;N	;C	;N	3CN
Syn_18	AUS30337	2=C	0	23C	3+	3+	3+	2+C	3+	3+
Syn_19	AUS30341	0	0;N	2C	;CN	0;C	0;	3C	0	11+CN
Syn_20	AUS30343	0	0;C	0;C	;1-C	3+	33+	33+	3+	3+
Syn_21	AUS30344	0	;CN	;N	2+C	3+	;N	0;	;CN	;N
Syn_22	AUS30353	0	0	23C	23C	;1-C	2-CN	;CN	1-N	0
Syn_23	AUS30357	0	0;	23C	23CN	0;C	2=N	;CN	3+	33+C
Syn_24	AUS30360	0;CN	;CN	1+C	2C	3+	2CN	0;	3+	;N+
Syn_25	AUS30398	0	;C	2+C	2CN	3+	;N	3+	3+	2=N
Syn_26	AUS30500	33+	3+	3+	3+	3+	3+	3+	3+	;CN
Syn_27	AUS30503	0;N+	;N+	;CN	;N	0;N+	;N	;CN	;N	;N
Syn_28	AUS30505	0;CN	;C	;C	;C	0;N+	;N	;CN	0;	;1-CN
Syn_29	AUS30508	0	0;	;CN	;CN	0;N+	;N	;CN	0;	33+CN
Syn_30	AUS30509	3+	3+	3+	33+	3+	3+	3+	3+	3+
Syn_31	AUS30511	0	0;	;C	;CN	0;C	;1-N	;CN	2CN	2++C
Syn_32	AUS30515	33+	3+	3+	33+	3+	3+	3+	3+	3+
Syn_33	AUS30521	0	;N	0;C	0;C	2+CN	;CN	;CN	0;	;N
Syn_34	AUS30525	0;CN	2-CN	3+	33+	3+	33+	22-C	3+	3+
Syn_35	AUS30538	0;	0;C	0;CN	;N	0;CN	0;	33+	0;	0;
Syn_36	AUS30542	0	2=CN	23C	23C	0	3+	;C	0	3+
Syn_37	AUS30547	0;C	2=CN	3C	3C	3+	3+	0	3+	3+
Syn_38	AUS30549	0;C	;N	0;C	2-C	1-C	2+C	;CN	;C	33+C
Syn_39	AUS30550	;C	;CN	0;C	33+	3+	3CN	;CN	;C	33+
Syn_40	AUS30552	0	0;CN	23CN	3CN	3+	3+	;CN	33+C	3+
Syn_41	AUS30553	0	0;C	0;C	2=C	;-CN	;CN	;CN	3+	;N
Syn_42	AUS30556	0	3+	3+	3+	3+	3+	;C	3+	3+
Syn_43	AUS30560	0	;C	0	;CN	3+	3C	0	3+	3+
Syn_44	AUS30562	0	2=C	33+	3+	0;C	3+	2-C	3+	3+
Syn_45	AUS30575	0	0;C	;1CN	;CN	22-C	2-N	2=C	3+	1+CN
Syn_46	AUS30577	33+	3+	3+	33+	33+	3+	3+	3+	3+
Syn_47	AUS30581	0	0;C	3+	33+	22-CN	2-N	;CN	2++CN	2++C
Syn_48	AUS30582	0;C	3+	3+	;CN	3+	3+	3+	3+	3-N
Syn_49	AUS30584	0;C	3+	3+	2CN	3+	1++N	3+	3+	3C
Syn_50	AUS30594	0;C	0	3+	2+CN	3+	2++N	3+	0;	2++N

Table 3 (continued)

Line ID	AUS No.	Pty1	Pty2	Pty3	Pty4	Pty5	Pty6	Pty7	Pty8	Pty9
Syn_51	AUS30596	0;C	0;	33+	3C	3+	2+C	22+CN	3C	2CN
Syn_52	AUS30611	0;CN	2++CN	;CN	;CN	3+	2-C	;CN	0;	3C
Syn_53	AUS30613	0;CN	;N	33+	;CN	33+C	;N	;CN	3C	33+C
Syn_54	AUS30615	0	0	3+	33+	3+	3+	3+	3+	33+C
Syn_55	AUS30617	0	0;C	2-CN	;N	0;C	2-CN	0;	3C	1-N
Syn_56	AUS30618	0;C	;N	3+	3+	23C	2N	2CN	2CN	3+
Syn_57	AUS30619	3+	33+	3+	33+	3+	3+	33+	3+	3+
Syn_58	AUS30620	0	0	3+	2C	3+	2-N	;+C	0;	33+CN
Syn_59	AUS30622	;1C	3+	22+CN	;CN	3C	3+	3+	3+	3
Syn_60	AUS30623	0	23-C	3+	3-C	3+	3+	3+	3+	3+
Syn_61	AUS30624	;CN	0;C	;CN	;CN	3+	2+C	;CN	3+	;N+
Syn_62	AUS30626	0	0;C	2=N+	0	22+N	;N	0;N	;N	;N+
Syn_63	AUS30632	3+	3+	33+	33+	33+	3+	3+	33+	;N
Syn_64	AUS30633	;CN	0	;N	;CN	0	;C	2CN	3C	1=CN
Syn_65	AUS30635	0	0	;C	0	0	0	0;	0;	2+CN
Syn_66	AUS30636	0	;C	;C	;-C	;1-CN	;CN	33+	3-C	2+CN
Syn_67	AUS30658	0	0	0;C	0;C	0	;N	0	3+	23C
Syn_68	AUS30664	0	0;	0;	0;C	0	3+	0;	3+	23C
Syn_69	AUS33376	3+	3+	3+	3+	3+	3+	3+	3+	3+
Syn_70	AUS33381	33+	33+	33+	3+	3+	3+	3+	3+	3+
Syn_71	AUS33383	0	;C	33+	3+	3C	33+	3+	3+	23C
Syn_72	AUS33386	;CN	3+	;C	;C	2=C	;1N	3+	3+	;CN
Syn_73	AUS33388	0	0	2+C	0	0	;CN	3+	;CN	2+CN
Syn_74	AUS33391	0	0	;N	;C	;N	2CN	0	2++	;CN
Syn_75	AUS33394	0	0;C	3+	33+	1=CN	2-N	;CN	0;	2+CN
Syn_76	AUS33395	0	3+	3+	;CN	3+	3+	3+	3+	3+
Syn_77	AUS33398	0	0;CN	3C	2+C	2CN	;CN	3+	3CN	;N
Syn_78	AUS33401	0	;C	2++C	0	0	;CN	;N	0	2C
Syn_79	AUS33402	0	0;C	;C	0	0	0;C	0;	;CN	23C
Syn_80	AUS33403	3+	3+	3+	3+	3+	3+	3+	3+	3+
Syn_81	AUS33406	33+	33+	3+	33+	33+	3+	3+	3+	33+C
Syn_82	AUS33408	0	0	;CN	3+	3+	;CN	;N	3+	2++CN
Syn_83	AUS33424	0	0	33+	3+	3+	3+	2-CN	0;N	1-C
Syn_84	AUS34091	3+	3+	3+	33+	3+	3+	3+	3+	3+
Syn_85	AUS34097	3+	3+	3+	3+	3+	3+	3+	3+	33+C
Syn_86	AUS34100	0;CN	;1CN	3+	;CN	0;CN	23CN	23C	;C	3+
Syn_87	AUS34116	0;C	;N	;C	;CN	0;C	;N	;CN	0;	;1-C
Syn_88	AUS34119	0;CN	3+	33+	23-C	0	3+	3+	33+	33+
Syn_89	AUS34123	0;CN	;CN	3-C	23C	3+	3+	3+	3+	3+
Syn_90	AUS34142	0;CN	3+	0	;CN	3+	3+	;CN	3+	3+
Syn_91	AUS34148	3+	3+	3C	3+	3+	3C	3+	3+	3+
Syn_92	AUS34169	3+	3+	3+	3+	3+	3+	3+	33+	33+
Syn_93	AUS34198	2+C	3+	;C	2-CN	2+CN	3+	3+	3+	33+CN
Syn_94	AUS34219	;1+CN	0;CN	3C	33+	0;CN	;N	3+	;C	3C
NIL	AvS + *Yr18*	3+	3+	3+	3+	3+	3+	3+	3+	3+

Table 3 (continued)

Yr gene/s	Diff. Line	Pty1	Pty2	Pty3	Pty4	Pty5	Pty6	Pty7	Pty8	Pty9
Yr1	Chinese 166	0	0	0;C	0	0;	0	3+	0	0;
Yr7	Lee	;CN	3+	3+	3+	3+	3+	3+	3+	3+
Yr2, *Yr6*	Heines Kolben	;N	3+	3+	3+	3+	3+	3+	3+	3+
Yr3	Vilmorin27	3+	3+	;N	;N	;CN	;N	3+	3+	;CN
Yr10	Moro	0	0	0	;C	;C	;C	0	0	33+C
YrSD	Strubes Dickkopf	33+C	;CN	0;C	;CN	;CN	;CN	3+	0	2=C
YrSU	Suwon 92/Omar	3+	33+C	0;C	0	2C	0	3+	3+	0
Yr2, *Yr9*	Clement	0	0	;N	3+	3+	3+	3+	;CN	0;C
Yr5	Triticum Spelta	0	0	0	0	0	0	0	0	0
Yr4	Hybrid 46	33+C	33+	0;CN	0;	0	0;	3+	2CN	;N
Yr7	Reichersberg 42	;N	3+	1-N	;N	33+N	;N	2++CN	2++CN	;N
Yr2, *Yr6*	Heines Peko	0	23-C	3+	3+	3+	3+	3+	;CN	3+
YrND	Nord Desprez	3+	3+	1N	;CN	;CN	;CN	3+	0;C	33+C
Yr8	Compair	0	0;	3+	3+	3+	3+	0	2=CN	0
Yr32	Carstens V	;C	;CN	0;	;N	;C	;N	3+	;CN	;CN
YrSP	Spaldings Prolific	;C	;C	0	0	;CN	0	3+	0;C	0
Yr2, *Yr25*	Heines VII	3+	33+C	0;C	3+	3+	3+	3+	0;C	2=C
YrA (*Yr73*, *Yr74*)	Avocet R	3+	3+	3+	3+	3+	3+	;CN	3+	3+
Yr2	Kalyansona	3+	3+	3+	3+	3+	3+	3+	3+	3+
Yr17	Trident	;N	0;C	;N	;N	3+	;N	3+	3+	3+
Yr15	NIL *Yr15*/6* AvS	0	0	0	0	0	0	0	0	0
Yr25	Hugenoot	3+	3+	3+	3+	3+	3+	3+	2=CN	3+
Yr27	Selkirk	33+CN	;CN	22+N	3+	3+	0;	2=N+	22+N	2CN
Yr9	NIL *Yr9*/Avs	0;	0	3+	3+	3+	3+	3+	3+	3+
Susc	Avocet S	3+	3+	3+	3+	3+	3+	3+	3+	3+
Yr33	EGA Gregory	0;CN	22+CN	2=CN	0;C	;1CN,3+	0;C	3+	0	1CN
Yr17	Ellison	0	0	0;CN	0	3+	0	0?	3+	3+
YrT	Tobruk	0	0	2=N+	;N	;N+	3+	2-N+	3+	2=N
YrJ	Breakwell	;CN	;C	2N+	3+	22+	3+	2++3+	3+	2=C
Yr17	NIL *Yr17*/Avs	;N	0;C	2CN	1C	3+	1C	3+	33+	;CN
Susc	Morocco	3+	3+	3+	3+	3+	3+	3+	3+	3+
Barley	Clipper Sahara	0	0	0;	0;C	0	0;C	0	3+	0;C

Pty Pathotype, *AvS* Avocet S, *NIL* Near Isogenic Line, *Susc*. Susceptible control

Pty1: 104 E137 A+, Pty2: 110 E143 A+, Pty3: 134 E16 A+, Pty4: 134 E16 A+ J+ Yr27+, Pty5: 134 E16 A+ 17+ 27+, Pty6: 134 E16 A+ J+ T+, Pty7: 239 E237 A- 17+ 33+, Pty8: 198 E16 A+ J+ T+ 17+, Pty9: 150 E16 A+

(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). The 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 the DNA pellet. Tubes were kept in an oven (37 °C) for 2 h 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).

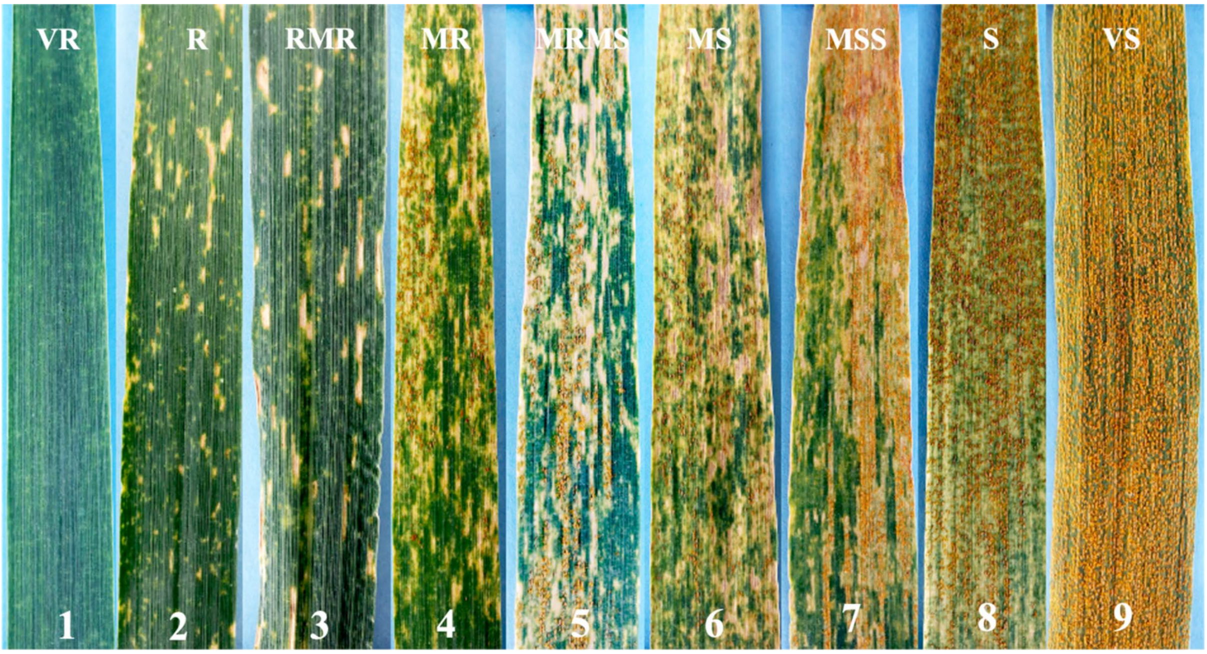

Fig. 1 Field stripe rust scoring scale (1–9). Source: Sandhu et al. (2021)

Genotyping using molecular markers

Core set and control lines were genotyped with three gel-based markers and one Kompetitive allele specific PCR (KASP) marker, each linked to a stripe rust resistance gene. The codominant microsatellite marker csLV34 (Lagudah et al. 2006) linked with *Yr18*, gene specific WKS1 and WKS2 markers (Fu et al. 2009; Huang et al. 2016) linked with *Yr36*, and KASP marker TM4 linked with *Yr46* (Moore et al. 2015) were used for genotyping. Details of all markers and PCR profiles used in this study are provided in Table 5.

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 5 × MyFi™ (Bioline Australia Pty. Ltd. Alexandria

Table 4 Description of 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)

Table 5 Oligonucleotide primers and their PCR profiles used for the amplification of markers linked with *Yr18*, *Yr36* (*WKS*) and *Yr46* genes

Gene	Size (bp)	Ann (ºC)	Ext (s)	Primer name	Primer sequence	Reference
Yr18	Present: 150 Absent: 229	60	30"	csLV34: F csLV34: R	5'GTTGGTTAAGACTGGTGATGG3' 5'TGCTTGCTATTGCTGAATAGT3'	Lagudah et al. (2006)
Yr36	WKS1: 871 WKS2: 537	70–65*	60"	WKS_540F WKS_544R	5'GGCCACACTGCAATACTATACC3' 5'CACAAATCCTGGCTGTGGAC3'	Fu et al. (2009)
Yr36	WKS1: null WKS2: 452	64–59*	30"	WKS2_628F WKS2_629R	5'ACGACATTGGAGGCGTTACTACGA3' 5'ACGAAGGACGCAATGCTGCTGA3'	Huang et al. (2016)
Yr46	KASP marker	65–57**	60"	TM4	[a]Allele 1 (5'GAAGGTGACCAAGTT CATGCT3') [b]Allele 2 (5'GAAGGTCGGAGTCAACGGATT3')	Moore et al. (2015)

Touchdown PCRs cycles annealing temperature decreasing by *(0.5 ºC) and **(0.8°C) per cycle

[a]Primer labelled with FAM

[b]Primer labelled with HEX

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 h. For staining, 2.0 µl of Gel-Red™ (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 assays were 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, USA)], 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).

Results

None of the 94 lines tested positive for markers WKS1 and WKS2 linked to *Yr36*, and KASP marker TM4 linked with *Yr46* (Fig. 2), indicating that they lacked these genes. Based on overall combined field rust screening tests, greenhouse multi-pathotype testing using nine Australian pathotypes of *Pst* (Table 3) and genotyping with APR genes linked markers (Table 5), the core set fell into seven categories (Fig. 3): uncatalogued APR (UAPR, 11%); uncatalogued seedling resistance (USR, 46%); known seedling resistance (KSR, 5%); KSR + USR (2%); *Yr18* + UAPR (4%); *Yr18* + USR (29%); and *Yr18* + KSR (3%).

Multi-pathotype seedling tests and marker analysis

Multi-pathotype testing with nine *Pst* pathotypes revealed that 14 lines (AUS30288, AUS30332, AUS30509, AUS30515, AUS30577, AUS33403, AUS33406, AUS34091, AUS34097, AUS34148, AUS30619, AUS33376, AUS33381, AUS34169) did not carry any detectable seedling resistance to *Pst* in Australia (Table 3). All 14 lines were resistant to *Pst* across all environments, and were hence concluded to carry uncatalogued (new)

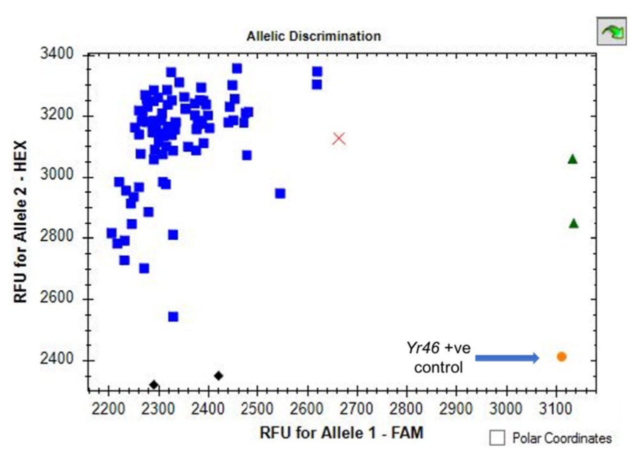

Fig. 2 Genotyping of core set using *Yr46* linked KASP marker CSTM4 did not produce any cluster for the presence of *Yr46* Allele 1 (SNP1, FAM) near the positive control and blue squares represented Allele 2 (SNP2, HEX) cluster

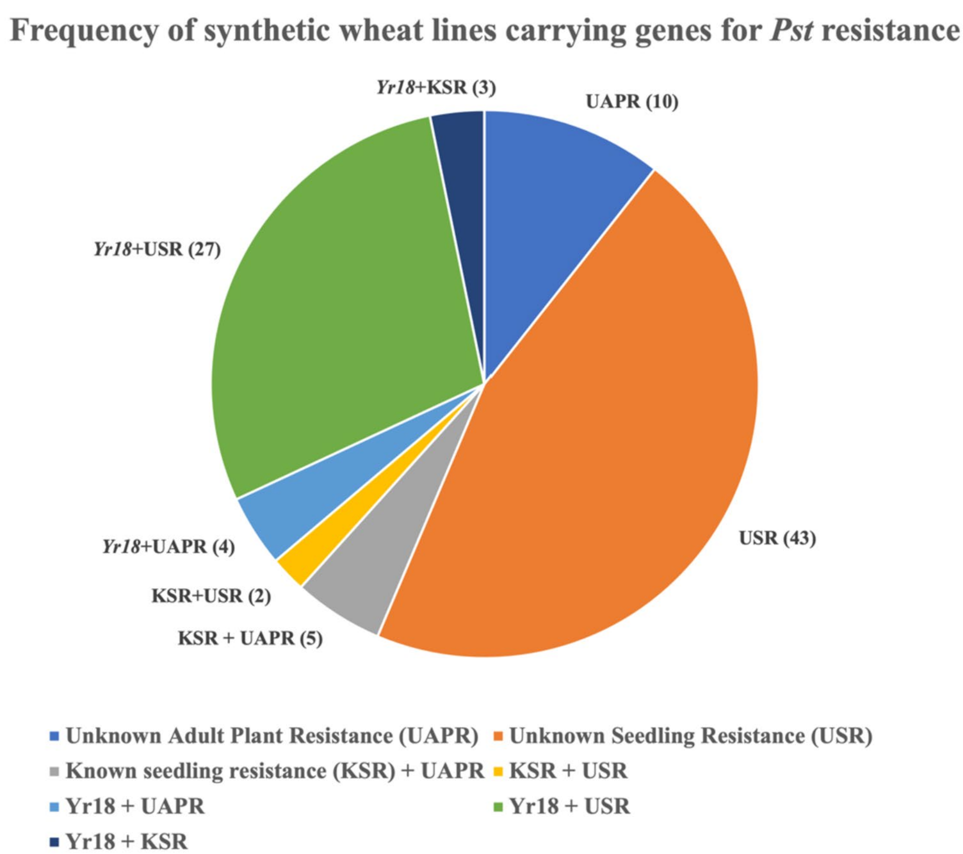

Fig. 3 Rust screening of core set lines under both greenhouse and field conditions and genotyping using markers linked with APR genes *Yr18*, *Yr36* and *Yr46*

APR (UAPR) to *Pst* that was effective under Australian conditions and possibly elsewhere (Table 6). Genotyping of these lines revealed that four (AUS30619, AUS33376, AUS33381, AUS34169) carried the *Yr18* linked marker. Given the higher resistant response of these four lines as compared to the NIL AvocetS + *Yr18*, these lines were concluded to carry additional uncatalogued APR (UAPR). Lines AUS30288, AUS30515 and AUS34097 were more resistant than the other lines carrying UAPR (Table 6).

Seventy lines were seedling resistant to all nine test pathotypes. These lines could carry one or more ASR gene(s) that are effective to all pathotypes (eg. *Yr15*) or uncatalogued ASR (Table 3). The resistance in these lines was highly effective in the field in Australia, and although this masked the potential presence of APR, marker genotyping indicated the presence of *Yr18* in all.

Line AUS30398 was susceptible to the *Yr17*-virulent pathotypes 134E16A+17+27+, 239E237A-17+33+, and 198E16A-J+T+17+, and resistant to all other six pathotypes used (Table 3), indicating that it likely carries *Yr17*. Similarly, the remaining nine lines (AUS30538, AUS30615, AUS30265, AUS30266, AUS30282, AUS30283, AUS30336, AUS30508, AUS30549) showed compatible infection types against at least one of the nine pathotypes and were postulated to carry known seedling resistance genes including *Yr1* or *Yr32* or *Yr33* (AUS30538), *Yr9* (AUS30615) and *Yr10* (AUS30265, AUS30266, AUS30282, AUS30283, AUS30336, AUS30508, AUS30549). Of the 70 lines carrying USR, 27 carried the *Yr18* linked marker (Table 7) and were also resistant in the field. Ten lines with no detectable ASR that lacked markers for *Yr18* and *Yr46* carried low to high levels of resistance in the field indicating that they likely carry APR distinct from *Yr18*. Another four lines (AUS30619, AUS33376, AUS33381and AUS34169) with no ASR in the background and carrying the *Yr18* linked marker were more resistant as compared to *Yr18* alone (Table 6), indicating that the UAPR carried by these lines is distinct from APR gene *Yr18*.

Multi-site field evaluation

The number of lines expressing high levels (VR–RMR) of resistance to *Pst* under field conditions varied across the different environments: Australia (92 lines), Ethiopia (30), India (63), Kenya (74), Nepal (48) and Pakistan (32)) (Fig. 4). The remaining lines expressed medium (MR-MS) to low levels (MS-S) of resistance to *Pst* that also varied between countries. The highest number of lines scored MR-MS occurred in Ethiopia (49 lines), followed by India (31), Nepal (17), Kenya (14), Pakistan (11) and Australia (2). A higher number of lines expressed low levels of *Pst* resistance under field conditions in Nepal and Pakistan, whereas only two and 14 lines were

Table 6 Field rust testing data across the environments from Australia, Ethiopia, India, Kenya, Nepal and Pakistan, recorded as per the 1 to 9 field rust scale

PBI#	AUS No.	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
Syn_1	AUS30265	2	2	2	2	2	3	2	1	1	2	1	1
Syn_2	AUS30266	2	1	2	2	3	2	2	1	1	1	1	1
Syn_3	AUS30268	3	3	3	3	3	3	2	1	1	2	1	1
Syn_4	AUS30269	1	1	1	1	1	3	2	1	1	1	6	6
Syn_5	AUS30273	2	2	3	3	3	6	2	1	1	1	1	1
Syn_6	AUS30275	2	1	2	2	1	2	2	1	1	7	2	3
Syn_7	AUS30279	2	1	2	2	2	6	2	1	1	2	5	4
Syn_8	AUS30282	2	2	2	2	1	3	2	1	1	1	1	1
Syn_9	AUS30283	2	1	2	2	1	1	2	1	1	2	1	1
Syn_10	AUS30288	3	3	3	3	3	1	3	1	1	1	1	1
Syn_11	AUS30307	2	1	2	2	3	6	3	1	1	7	7	7
Syn_12	AUS30311	2	2	3	3	3	7	3	1	1	2	7	7
Syn_13	AUS30312	2	2	2	2	1	7	2	2	1	2	1	1
Syn_14	AUS30320	2	2	2	2	2	2	3	1	1	2	2	2
Syn_15	AUS30332	2	2	3	3	3	5	4	5	5	6	7	7
Syn_16	AUS30334	3	3	3	3	3	7	3	2	2	7	7	7
Syn_17	AUS30336	2	2	2	2	1	4	2	1	1	1	1	1
Syn_18	AUS30337	2	3	3	3	2	2	5	2	2	2	7	7
Syn_19	AUS30341	2	3	3	3	1	6	3	1	1	6	1	1
Syn_20	AUS30343	2	1	2	2	1	2	5	1	1	2	7	7
Syn_21	AUS30344	2	3	3	3	2	6	4	6	6	2	2	2
Syn_22	AUS30353	2	2	2	2	1	6	2	1	1	2	3	4
Syn_23	AUS30357	3	2	2	2	1	4	2	1	1	1	7	7
Syn_24	AUS30360	2	1	2	2	1	7	2	2	2	5	7	7
Syn_25	AUS30398	2	2	2	2	1	6	2	1	1	1	7	7
Syn_26	AUS30500	3	2	2	2	2	3	4	5	5	7	7	7
Syn_27	AUS30503	3	2	3	3	2	3	4	2	2	5	7	7
Syn_28	AUS30505	3	2	2	2	1	3	2	2	2	1	2	1
Syn_29	AUS30508	2	1	2	2	1	3	4	1	1	3	1	1
Syn_30	AUS30509	2	2	3	3	3	3	2	1	1	7	7	7
Syn_31	AUS30511	2	2	2	2	1	6	2	1	1	7	1	1
Syn_32	AUS30515	2	3	3	3	3	2	2	3	3	3	2	2
Syn_33	AUS30521	2	3	2	2	1	7	2	3	3	6	2	3
Syn_34	AUS30525	2	2	2	2	1	2	2	1	1	6	7	7
Syn_35	AUS30538	2	2	2	2	1	6	2	1	1	1	1	1
Syn_36	AUS30542	3	2	2	2	1	5	2	5	5	7	1	1
Syn_37	AUS30547	2	3	3	3	3	6	2	3	3	6	7	7
Syn_38	AUS30549	2	2	3	3	2	4	2	3	3	3	3	3
Syn_39	AUS30550	3	2	2	2	2	6	2	5	5	1	7	7
Syn_40	AUS30552	2	2	2	2	1	6	2	3	3	5	3	4
Syn_41	AUS30553	2	1	1	1	1	5	5	3	2	2	7	7
Syn_42	AUS30556	3	2	3	3	2	4	5	2	2	2	7	7
Syn_43	AUS30560	3	3	3	3	3	6	4	6	6	2	2	1
Syn_44	AUS30562	2	2	2	2	2	6	3	3	3	2	7	7
Syn_45	AUS30575	2	2	2	2	2	6	4	2	2	2	7	7
Syn_46	AUS30577	3	2	2	2	2	5	2	5	5	7	7	7
Syn_47	AUS30581	2	3	5	5	1	4	5	1	1	5	7	7
Syn_48	AUS30582	3	2	2	2	2	6	6	6	6	5	7	7
Syn_49	AUS30584	2	2	2	2	1	7	5	6	7	6	7	7
Syn_50	AUS30594	2	2	2	2	1	7	2	1	1	1	7	7
Syn_51	AUS30596	2	2	2	2	2	6	3	2	2	1	7	7

Table 6 (continued)

PBI#	AUS No.	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
Syn_52	AUS30611	2	2	2	2	1	6	2	6	6	7	2	1
Syn_53	AUS30613	2	2	3	3	1	1	2	3	2	6	7	7
Syn_54	AUS30615	2	1	2	2	1	4	2	2	2	2	2	2
Syn_55	AUS30617	2	2	2	2	2	3	2	5	5	5	2	1
Syn_56	AUS30618	3	3	3	3	3	3	5	2	1	7	5	6
Syn_57	AUS30619	2	2	3	3	2	3	3	1	1	6	7	7
Syn_58	AUS30620	3	2	2	2	2	6	3	5	6	2	7	7
Syn_59	AUS30622	2	2	2	2	2	7	2	1	1	1	1	1
Syn_60	AUS30623	5	5	6	5	5	5	2	5	5	7	7	7
Syn_61	AUS30624	2	2	2	2	2	7	6	5	5	2	7	7
Syn_62	AUS30626	2	3	3	3	3	7	2	1	1	1	2	1
Syn_63	AUS30632	2	2	2	2	2	2	4	2	2	2	7	7
Syn_64	AUS30633	3	3	2	2	2	7	2	2	2	7	7	7
Syn_65	AUS30635	2	1	2	2	2	5	2	1	1	1	1	2
Syn_66	AUS30636	3	3	3	3	3	1	2	2	2	7	7	7
Syn_67	AUS30658	2	3	3	3	2	5	2	1	1	7	7	7
Syn_68	AUS30664	2	2	2	2	2	6	2	1	1	7	7	7
Syn_69	AUS33376	2	2	2	2	2	5	5	5	5	7	7	7
Syn_70	AUS33381	3	3	2	2	2	5	2	1	1	7	7	7
Syn_71	AUS33383	2	3	3	3	3	5	3	1	1	1	7	7
Syn_72	AUS33386	3	3	3	3	3	7	4	5	5	7	7	7
Syn_73	AUS33388	2	2	2	2	2	6	4	1	1	2	2	2
Syn_74	AUS33391	2	2	2	2	1	5	2	1	1	7	1	2
Syn_75	AUS33394	2	2	3	3	2	3	4	1	1	2	7	7
Syn_76	AUS33395	2	3	3	3	3	7	3	5	5	7	7	7
Syn_77	AUS33398	3	3	3	3	2	6	2	1	1	7	3	4
Syn_78	AUS33401	3	3	3	3	3	2	4	3	3	1	7	7
Syn_79	AUS33402	2	3	3	3	3	7	5	2	2	1	1	1
Syn_80	AUS33403	3	3	3	3	3	3	4	2	3	7	7	7
Syn_81	AUS33406	2	3	3	3	2	3	5	2	2	7	7	7
Syn_82	AUS33408	5	5	5	5	5	6	4	5	5	2	7	7
Syn_83	AUS33424	3	3	3	3	3	6	4	1	1	1	7	7
Syn_84	AUS34091	3	3	3	3	3	5	5	2	2	7	6	6
Syn_85	AUS34097	3	3	3	3	2	3	3	2	2	2	3	3
Syn_86	AUS34100	2	2	2	2	2	6	4	3	3	7	7	7
Syn_87	AUS34116	2	2	2	2	1	6	3	1	1	5	2	1
Syn_88	AUS34119	2	3	3	3	3	3	2	7	7	7	6	7
Syn_89	AUS34123	2	1	1	1	1	6	3	6	6	2	7	7
Syn_90	AUS34142	2	2	2	2	2	6	5	2	2	6	6	6
Syn_91	AUS34148	2	2	2	2	2	3	4	3	3	7	7	7
Syn_92	AUS34169	3	3	5	5	5	5	3	3	3	2	1	1
Syn_93	AUS34198	2	1	2	1	2	5	3	3	2	2	6	5
Syn_94	AUS34219	3	3	3	3	2	3	3	2	2	5	6	6
NIL	AvS + *Yr18*	7	7	7	7	7	7	7	7	7	7	7	7
Susc	Avocet S	9	9	9	9	9	9	9	9	9	9	9	9

E1: Australia 2016, E2: Australia 2017, E3: Australia 2018, E4: Australia 2019, E5: Australia 2020, E6: Ethiopia 2020, E7: India 2020, E8: Kenya 2019, E9: Kenya 2020, E10: Nepal 2020, E11: Pakistan (Islamabad) 2020 and E12: Pakistan (Nowshehra) 2020

E Environment, *AvS* Avocet S, *NIL* Near Isogenic Line, *Susc.* Susceptible control

Table 7 Postulation of genes for *Pst* using field rust testing, greenhouse multi-pathotype testing and molecular markers genotyping data

PBI#	AUS No.	Postulated gene/s for *Pst* resistance
Syn_1	AUS30265	*Yr10*+*Yr18*
Syn_2	AUS30266	*Yr10*+*Yr18*
Syn_3	AUS30268	*Yr18*+USR
Syn_4	AUS30269	USR
Syn_5	AUS30273	USR
Syn_6	AUS30275	USR
Syn_7	AUS30279	USR
Syn_8	AUS30282	*Yr10*+UAPR
Syn_9	AUS30283	*Yr10*+UAPR
Syn_10	AUS30288	UAPR
Syn_11	AUS30307	USR
Syn_12	AUS30311	USR
Syn_13	AUS30312	USR
Syn_14	AUS30320	USR
Syn_15	AUS30332	UAPR
Syn_16	AUS30334	USR
Syn_17	AUS30336	*Yr10*+UAPR
Syn_18	AUS30337	USR
Syn_19	AUS30341	*Yr18*+USR
Syn_20	AUS30343	USR
Syn_21	AUS30344	*Yr18*+USR
Syn_22	AUS30353	USR
Syn_23	AUS30357	USR
Syn_24	AUS30360	USR
Syn_25	AUS30398	*Yr17*+USR
Syn_26	AUS30500	USR
Syn_27	AUS30503	USR
Syn_28	AUS30505	USR
Syn_29	AUS30508	*Yr10*+UAPR
Syn_30	AUS30509	UAPR
Syn_31	AUS30511	*Yr18*+USR
Syn_32	AUS30515	UAPR
Syn_33	AUS30521	USR
Syn_34	AUS30525	USR
Syn_35	AUS30538	*Yr18*+*Yr1*/*Yr32*/*Yr33*
Syn_36	AUS30542	USR
Syn_37	AUS30547	USR
Syn_38	AUS30549	*Yr10*+UAPR
Syn_39	AUS30550	*Yr18*+USR
Syn_40	AUS30552	USR
Syn_41	AUS30553	*Yr18*+USR
Syn_42	AUS30556	*Yr18*+USR
Syn_43	AUS30560	*Yr18*+USR
Syn_44	AUS30562	USR
Syn_45	AUS30575	USR
Syn_46	AUS30577	UAPR
Syn_47	AUS30581	USR
Syn_48	AUS30582	*Yr18*+USR
Syn_49	AUS30584	USR
Syn_50	AUS30594	USR
Syn_51	AUS30596	USR
Syn_52	AUS30611	USR
Syn_53	AUS30613	USR
Syn_54	AUS30615	*Yr9*+USR
Syn_55	AUS30617	*Yr18*+USR
Syn_56	AUS30618	*Yr18*+USR
Syn_57	AUS30619	*Yr18*+UAPR
Syn_58	AUS30620	USR
Syn_59	AUS30622	*Yr18*+USR
Syn_60	AUS30623	*Yr18*+USR
Syn_61	AUS30624	*Yr18*+USR
Syn_62	AUS30626	USR
Syn_63	AUS30632	USR
Syn_64	AUS30633	USR
Syn_65	AUS30635	*Yr18*+USR
Syn_66	AUS30636	USR
Syn_67	AUS30658	*Yr18*+USR
Syn_68	AUS30664	*Yr18*+USR
Syn_69	AUS33376	*Yr18*+UAPR
Syn_70	AUS33381	*Yr18*+UAPR
Syn_71	AUS33383	USR

UAPR Unknown adult plant resistance, *USR* Unknown seedling resistance

recorded in this category in Kenya and Ethiopia, respectively (Fig. 4). No line expressed low levels of resistance in Australia or in India (Karnal).

Eight lines (AUS30265, AUS30266, AUS30268, AUS30282, AUS30283, AUS30288, AUS30320 and AUS30505) were scored as VR–RMR (1–3) and 17 lines (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 and all years tested (Table 8).

Discussion

Continued evolution in *Pst* globally has resulted in the emergence of virulence for many of the known and deployed ASR genes along with previously detected virulences for other ASR genes (*eg Yr1*, *Yr2*, *Yr3*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr19*, *Yr20*, *Yr21*, *Yr22*, *Yr23*, *Yr24*, *Yr25*, *Yr26*, *Yr27*, *Yr28*, *Yr31*, *Yr32*, *Yr33*, *Yr35*, *Yr43*, *Yr44*, *Yr76*; Calonnec and Johnson 1998; Chen 2005; Chen et al. 2010; Holtz et al. 2013; Kumar

Fig. 4 Rust response of core set lines tested across the environments

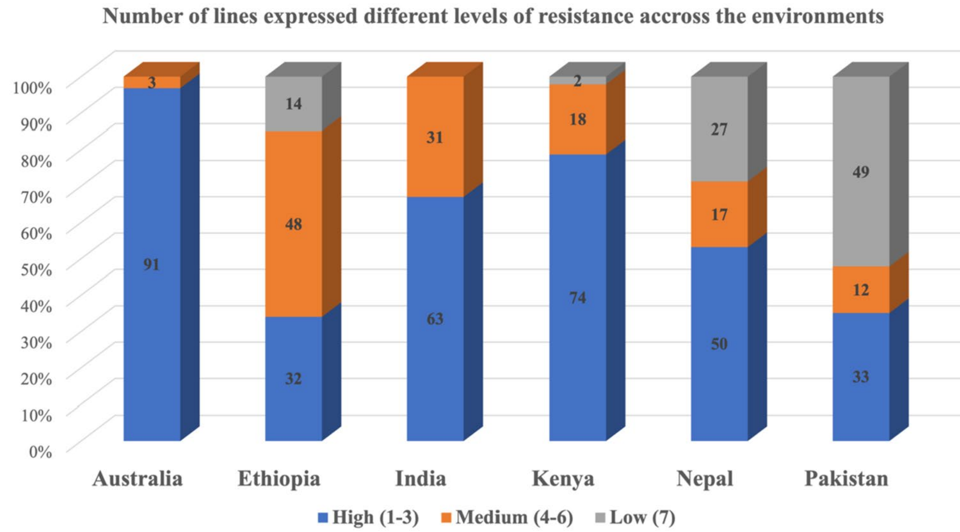

Table 8 Lines which expressed medium to high levels of resistance against *Puccinia striiformis* f. sp. *tritici* when tested under field conditions across the countries

PBI#	AUS No. ID	Gene Postulation	Level of protection
Syn_1	AUS30265	*Yr18*+USR	High
Syn_2	AUS30266	*Yr18*+USR	High
Syn_3	AUS30268	*Yr18*+USR	High
Syn_8	AUS30282	USR	High
Syn_9	AUS30283	USR	High
Syn_10	AUS30288	UAPR	High
Syn_14	AUS30320	USR	High
Syn_28	AUS30505	USR	High
Syn_32	AUS30515	UAPR	High
Syn_85	AUS34097	UAPR	High
Syn_4	AUS30269	USR	Medium
Syn_5	AUS30273	USR	Medium
Syn_7	AUS30279	USR	Medium
Syn_17	AUS30336	USR	Medium
Syn_19	AUS30341	*Yr18*+USR	Medium
Syn_22	AUS30353	USR	Medium
Syn_35	AUS30538	*Yr18*+*Yr1*/*Yr32*/*Yr33*	Medium
Syn_40	AUS30552	USR	Medium
Syn_54	AUS30615	USR	Medium
Syn_55	AUS30617	*Yr18*+USR	Medium
Syn_65	AUS30635	*Yr18*+USR	Medium
Syn_73	AUS33388	*Yr18*+USR	Medium
Syn_87	AUS34116	USR	Medium
Syn_90	AUS34142	*Yr18*+USR	Medium
Syn_93	AUS34198	*Yr18*+USR	Medium
Syn_94	AUS34219	USR	Medium

High: 1–3 score, and Medium: 4–6 score as per 1–9 scale (Fig. 1)

et al. 1988; Line and Qayoum 1992; Liu et al. 2016; McIntosh et al. 1995; Park et al. 2020; Sharma-Poudyal et al. 2013; Stubbs 1985; Wan and Chen 2012, 2014; Wan et al. 2004, 2016, 2017; Wellings and Burdon 1992; Wellings et al. 2009; Yang et al. 2013) and also combined virulence for important gene combinations (eg. *Yr17* plus *Yr33*; Park et al. 2020). This highlights the importance of finding and deploying new durable sources of resistance to manage WYR. Synthetic hexaploid wheats developed by crossing *A. tauschii* with tetraploid wheat have been considered an important means of introducing new genetic diversity into the hexaploid wheat germplasm pool to combat abiotic and biotic stresses (Börner et al. 2015; Kishii et al. 2019). The present study targeted a collection of such synthetic hexaploid wheats to search for new sources of resistance to *Pst*.

We initially tested a set of 766 synthetic wheat lines for responses to Australian pathotypes of *Pst*. All lines were tested as seedlings under greenhouse conditions for ASR and for three years in Australia in field trials that were artificially inoculated with defined pathotypes of *Pst*. A core set was identified as carrying APR under Australian conditions based on field responses and seedling tests of ASR with nine Australian pathotypes of *Pst*. Characterising the ASR in the lines was important as it allowed the identification of lines carrying APR under Australian conditions. Comparative analyses of results from both greenhouse multi-pathotype testing and field-testing revealed the presence of APR in 14 lines AUS30288, AUS30332, AUS30509, AUS30515, AUS30577, AUS30619, AUS33376, AUS33381, AUS33403, AUS33406, AUS34091, AUS34097, AUS34148, and AUS34169, all of which did not carry any seedling resistance against any of the nine Australian pathotypes of *Pst* but were resistant in the field in all countries. While four of these lines were found to carry *Yr18*, it is likely that the remaining 10 carry uncatalogued new APR that is effective across the environments of eastern Africa, Oceania and south Asia. Exceptionally, three lines (AUS30288, AUS30515 and AUS34097) were found to be highly resistant across the field environments with a maximum of 1–3 field rust scores and it is predicted that these lines likely carry a combination of two or more resistance loci providing high levels of broadly effective APR. A combination of an APR gene *Yr62* and a slow rusting QTL has been reported to provide high levels of APR to stripe rust at high temperature in spring wheat line PI 192252 (Lu et al. 2014). Similarly, Uauy et al. (2005) reported that wheat breeding lines developed from the backcrossing of *Yr36* source 'Glupro' with 'Anza', and carrying both APR genes *Yr18* and *Yr36*, expressed high levels of WYR resistance in comparison to genotypes carrying *Yr18* alone. Lines AUS30288, AUS30515 and AUS34097 detected in this study represent useful sources of broadly effective APR for stripe rust for wheat breeding programs of countries in eastern Africa, Oceania and south Asia.

Four lines AUS30619, AUS33376, AUS33381 and AUS34169 carrying a combination of the *Yr18* linked marker and UAPR expressed high to medium levels resistance across the environments, but lines AUS30619, AUS33376 and AUS33381 expressed low levels of resistance in Nepal and Pakistan. As *Yr18* alone (NIL Avocet S+*Yr18*) provided low levels of resistance across all the environments, the expression of high to medium levels resistance in these four lines is most likely derived from the additive effect of *Yr18* with the UAPR present in each. Previously, Singh et al. (2011) achieved high levels of stripe rust resistance by combining APR genes in different spring wheats. They advocated the pyramiding of 4–5 APR genes for accomplishing durable resistance against both stripe rust and leaf rust in wheat. In this context, synthetic wheat lines AUS30619, AUS33376, AUS33381, and AUS34169 carrying a combination of *Yr18* and UAPR are useful sources for attaining durable stripe rust resistance.

So far, only one APR gene *Yr18*, which was derived from an Asian wheat landrace, has been used widely in modern wheat cultivars (McIntosh et al. 1995; Kolmer et al. 2008). Another APR gene *Yr46*, though widely distributed in wheat landraces in India and Pakistan (Moore et al. 2015) is not commonly used in the modern wheat cultivars grown in this region. Consequently, there is a dearth of APR genes for stripe rust resistance and the sources identified in this study are important contributors to fill this gap. In addition to the rust resistance, these synthetic wheat lines can also be used to improve much needed genetic diversity for other traits in bread wheat. Because the bulk of synthetic wheat lines characterised to carry UAPR or USR either singly or in combination with *Yr18* showed high levels of resistance against *Pst* across the field conditions of Australia, Ethiopia, India, Nepal, and Pakistan, the lines represent an important source of new broadly effective stripe rust resistance for wheat improvement globally.

Authors contributions Karanjeet S. Sandhu planned and conducted all the greenhouse, field, and lab experiments, and drafted this manuscript. Robert F. Park conceived the project and secured funding, and along with Davinder Singh provided valuable suggestions in writing this manuscript. Fikrte Y. Belayineh, Tamrat Negash, Hanif Khan, Subhash C. Bhardwaj, Suraj Baidya, Dhruba B. Thapa, Muhammad Fayyaz, Shahzad Asad and Mandeep S. Randhawa helped in the field rust screening of germplasm.

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Data availability statement The authors confirm that the research data and supporting information on this study are available within the article and in the form of its supplementary files. Seed of these lines can be provided on request and under SMTA.

Declarations

Conflict of interest Authors declare that there is no conflict of interests exist in relation to this study.

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