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Assessment of Genetic Diversity for Stem Rust and Stripe Rust Resistance in an International Wheat Nursery Using Phenotypic and Molecular Technologies

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Abstract. The objective of this study was to assess diversity for stem rust and stripe rust resistance in an international wheat screening nursery under greenhouse conditions using pathotypes with known avirulence/ virulence profiles. A set of 95 entries of an international wheat screening nursery collected from material generated by staff of the International Maize and Wheat Improvement Centre (CIMMYT) was tested against seven Australian Pgt and five Pst pathotypes through artificial inoculation under the greenhouse conditions using standard procedures. Ten all-stage stem rust resistance genes (*Sr8a, Sr8b, Sr9b, Sr12, Sr17, Sr23, Sr24, Sr30, Sr31* and *Sr38*) and seven all-stage stripe rust resistance genes (*Yr3, Yr4, Yr6, Yr9, Yr17, Yr27* and *Yr34*) were postulated either singly or in combinations based on seedling responses of test entries against pathotypes differing in virulence for commonly deployed genes. *Sr30* and *Sr38* were the most common stem rust resistance genes in this nursery. The *Sr38*-linked stripe rust resistance gene *Yr17* was present in high proportion. The presence of rust resistance genes *Sr24, Sr31/Yr9, Sr38/Yr17* and *Yr4* were confirmed using the closely linked molecular markers. The adult plant resistance (APR) genes *Sr2* and *Lr34/Yr18/Sr57* were detected using linked molecular markers *csSr2* and *csLV34*, respectively. Genotypes carrying combinations of stem rust and stripe rust resistance were identified for use as donor sources in breeding programs.

Keywords: Gene, Host resistance, Stem rust, Stripe rust, Wheat.

Introduction

Rust diseases of wheat are among the most important production constraints in all wheat growing regions globally (McIntosh *et al.,* 1995; Roelfs *et al.,* 1992). Stem rust caused by *Puccinia graminis* f. sp. *tritici* (Pgt) and stripe rust caused by *P. striiformis* f. sp. *tritici* (Pst) can cause up to 100% yield loss on susceptible cultivars (Roelfs *et al.,* 1992; McIntosh *et al.,* 1995; Chen, 2005; Bansal *et al.,* 2014). Frequent emergence and rapid spread of more virulent and aggressive pathotypes of Pgt (Pretorius *et al.,* 2000; Nazari *et al.,* 2009) and Pst (Ali *et al.,* 2017, Chen, 2020; Chen *et al.,* 2014; Wellings, 2011; FAO, 2014) continue to pose a serious threat to global food

security (Singh *et al.,* 2011a, Chen, 2020). The emergence of highly virulent pathotypes of Pgt in the East African highlands combines unique and complex virulence defeating many resistance genes previously effective against local Pgt pathotypes in individual geographies (Vikram *et al.,* 2021). The detection of highly aggressive Pgt pathotypes in Uganda in 1999 (Ug99) rendered more than 80% of global wheat varieties susceptible (Singh *et al.,* 2011a). Over the past decade 14 variants within the Ug99 race group have emerged and spread across East African countries (Singh *et al.,* 2011a, Bhavani *et al.,* 2019). The rapid evolution, spread and aggressive nature of new Pst pathotypes in major wheat growing countries around the world has made it the most significant disease (Ali *et al.,* 2017, Vikram *et al.,* 2021). Stripe rust has historically been endemic to areas with humid and cool summers or in warm high-altitude areas with cool nights but in recent years, stripe rust has shown greater adaptation in warmer areas, where the disease was previously less important (Ali *et al.,* 2017, Vikram *et al.,* 2021). Repeated incursions of new pathotypes at national or continental scales have been reported (Ali *et al.,* 2014, Markell *et al.,* 2008, Walter *et al.,* 2016). High rates of mutation from avirulence to virulence (Hovmøller and Justesen, 2007) have contributed to increased susceptibility of varieties over area and time (Milus *et al.,* 2015, Sørensen *et al.,* 2014, Vikram *et al.,* 2021).

Various control options are available to minimize losses caused by rust pathogens. Fungicides effectively control stem rust (Wanyera *et al.,* 2009; Tadesse *et al.,* 2010; Macharia *et al.,* 2013) and stripe rust (Carmona *et al.,* 2020; Wellings, 2011; Murray and Brennan, 2009). The global cost of controlling wheat stripe rust using fungicides is at least $US 1 billion annually (Chen, 2020). The use of fungicides in Australia reduced losses from stripe rust by A$359 million, annually (Murray and Brennan, 2009). In China, about 6 million hectares of wheat are treated with fungicides (Kang *et al.,* 2010; Carmona *et al.,* 2020), while in the US Pacific Northwest, wheat growers spend at least $US 10 million on use of fungicides to control stripe rust every year (Chen, 2020). This is an expensive method of rust control; especially for small scale farmers worldwide (Oliver, 2014). Host plant resistance is the most effective, economical and eco-friendly method of controlling wheat rust diseases (Bariana *et al.,* 2007a; Qamar *et al.,* 2008; Vanzetti *et al.,* 2011; Bansal *et al.,* 2015). Long term success in breeding for triple rust resistance is influenced by knowledge of pathotypic evolution, availability of genetically diverse sources of natural resistance, and the access to high throughput screening methodologies (Bariana *et al.,* 2007a; Singh *et al.,* 2011a).

Knowledge of genetic basis of host resistance in wheat cultivars, high throughput screening and a well-developed pre-breeding pipelines form the basis for successful breeding (Bariana, 2003; Bariana *et al.,* 2007a; Admassu *et al.,* 2012; Bansal *et al.,* 2015). Host resistance is categorized into two types; seedling resistance also called all stage resistance (ASR) and adult plant resistance (APR) (Bariana, 2003; Chen, 2005; Bariana *et al.,* 2007a; Kou and Wang, 2010; Ellis *et al.,* 2014). ASR is controlled by genes with major effects, and it is often short lived as it is prone to be matched by evolution of virulence in pathogen populations. Durability of this resistance can be achieved by pyramiding more than two genes in new cultivars (Bariana *et al.,* 2007a; Bernardo *et al.,* 2012; Ellis *et al.,* 2014). On the other hand, APR is controlled by genes with small effects that express at the post seedling stages (Bariana, 2003). A combination of more than two APR genes is essential to achieve acceptable levels of resistance (Bariana and McIntosh, 1995; Singh et al. 2011b). Deployment of combinations of 4-5 APR genes confers 'near-immune' resistance and lasts for a longer time (Singh *et al.,* 2011b; Singh *et al.,* 2014). Hence, achievement of durable control of wheat rust diseases requires identification, characterization and deployment of combinations of diverse sources of resistance (Kolmer *et al.,* 2007; Bariana *et al.,* 2007a, 2007b; Admassu *et al.,* 2012). Advances in molecular marker technology and availability of gene-linked or gene-specific markers ensure efficient pyramiding

of rust resistance genes (Kolmer *et al.,* 2013). Molecular markers have been developed for several rust resistance genes (http://maswheat.ucdavis.edu/Index.htm). These markers can be used for the detection of target genes in germplasm collections in the absence of appropriate pathogen isolates.

Tests with an array of pathotypes differing in virulence genes offer the most efficient way to determine the genetic diversity for resistance to a target plant pathogen among a set of germplasm (Singh *et al.,* 2014). Interpretation of results from multi-pathotype testing is based on the gene-for-gene concept in the case of rust diseases. Resistance genes are postulated by comparing infection types (ITs) produced by an array of pathotypes on genotypes under consideration with ITs produced by genotypes carrying known resistance gene(s) (Pathan and Park, 2007; Singh *et al.,* 2008a, Singh *et al.,* 2014). This methodology has been widely used to postulate all stage rust resistance genes in wheat. It requires well characterized pathotypes with diverse combinations of virulence and avirulence profiles and such resources are available in several laboratories (Kolmer, 2003; Pathan and Park, 2007; Admassu *et al.,* 2012; Singh *et al.,* 2014). Field testing of seedling susceptible genotypes enables identification of genotypes carrying APR.

Wheat cultivars derived from the CIMMYT germplasm are grown globally through continuous exchange of material with national research programs (Singh and Rajaram, 2002; Ortiz *et al.,* 2008; Pretorius *et al.,* 2015). CIMMYT breeders incorporate diverse rust resistance genes into elite germplasm. The rust resistant lines with good agronomic traits are compiled into screening nurseries and distributed annually for rust screening in many wheat growing countries (Ortiz *et al.,* 2008). Although wheat lines distributed globally by CIMMYT are selected based on their resistance to the three rust diseases (Singh *et al.,* 2008b), screening of germplasm against the local rust flora is essential. This study was planned to test an international wheat screening nursery against several Australian Pgt and Pst pathotypes in the greenhouse to understand genetic diversity for stem rust and stripe rust resistance.

Materials and Methods

Host materials

A set of 95 lines from a CIMMYT (C21SAWYT-AUS) wheat screening nursery was tested in the greenhouse to postulate stem rust and stripe rust resistance genes. Pedigree details are listed in Table 1. Stem rust and stripe rust differential sets with known resistance genes were sown with each experiment. Details of the differentials used are listed in McIntosh *et al.,* (1995).

Pathogen materials

Wheat genotypes were tested with seven Australian Pgt pathotypes: 34-1,2,3,4,5,6,7 (Culture number 103); 34-1,2,3,6,7,(8),9 (205); 34-1,2,3,5,7,8,9 (206); 343-1,2,3,5,6,(8),9 (465); 98-1,2,(3),(5),6 (279); 34-1,2,7+*Sr38* (565); 34-2,4,5,7,11 (99) and five Pst pathotypes: 134 E16A+*Yr17*+ (599); 134 E16A+*Yr17*+*Yr27* (617); 110 E143A+ (444); 108 E141A+ (420), 104 E137A+ (414). The avirulence/virulence formulae of different pathotypes used are presented in Table 2.

Table 1. Pedigree information, postulated genes and molecular marker data for the 95 entries of an international wheat nursery

QCode	Pedigrees	Genes postulated		*Yr4*	*Lr24/Sr24*	*Lr26/Yr9/Sr31*	*Lr34/Yr18/Sr57*	*Lr37/Yr17/Sr38*	*Sr2*
		Stripe rust	Stem rust	*barc75*	*Sr24#12*	*iag95*	*csLV34*	*Ventriup+LN2*	*csSr2*
1:ZWW12	TOB/ERA//TOB/CNO67/3/PLO/4/VEE#5/5/KAUZ/6/FRET2/7/SERI*3//RL6010/4*YR/3/PASTOR/4/BAV92	*Yr3, ?*	*Sr12, Sr8a, Sr17, Sr30*	-	-	-	+	-	-
2:ZWW12	TOB/ERA//TOB/CNO67/3/PLO/4/VEE#5/5/KAUZ/6/FRET2/7/PAURAQ	*Yr3, ?*	*Sr8a, Sr17, Sr30*	-	-	-	+	-	-
3:ZWW12	TOB/ERA//TOB/CNO67/3/PLO/4/VEE#5/5/KAUZ/6/FRET2/7/PAURAQ	*Yr?*	*Sr8a, Sr17, Sr30*	-	-	-	+	-	-
4:ZWW12	NS-732/HER/3/PRL/SARA//TSI/VEE#5/4/FRET2/5/SERI*3//RL6010/4*YR/3/PASTOR/4/BAV92	*Yr27*	*Sr30, Sr23, Sr8a*	-	-	-	-	-	-
5:ZWW12	NS-732/HER/3/PRL/SARA//TSI/VEE#5/4/FRET2/5/PAURAQ	*Yr27*	*Sr30*	-	-	-	-	-	-
8:ZWW12	KA/NAC//TRCH/5/ESDA//ALTAR 84/AE.SQUARROSA (211)/3/ESDA/4/CHOIX	*Yr17, Yr27*	*Sr30, Sr8a, Sr17, Sr38*	-	-	-	-	+	-
9:ZWW12	KA/NAC//TRCH/5/ESDA//ALTAR 84/AE.SQUARROSA (211)/3/ESDA/4/CHOIX	*Yr17*	*Sr12, Sr38+*	-	-	-	-	+	-
10:ZWW12	KA/NAC//TRCH/5/ESDA//ALTAR 84/AE.SQUARROSA (211)/3/ESDA/4/CHOIX	*Yr17*	*Sr8a, Sr17, Sr30, Sr38*	-	-	-	-	+	-
11:ZWW12	KA/NAC//TRCH/4/MILAN/KAUZ//DHARWAR DRY/3/BAV92	*Yr17*	*Sr8a, Sr12, Sr17, Sr30, Sr38*	-	-	-	-	+	-

QCode	Pedigrees	Genes postulated		*Yr4*	*Lr24/Sr24*	*Lr26/Yr9/Sr31*	*Lr34/Yr18/Sr57*	*Lr37/Yr17/Sr38*	*Sr2*
		Stripe rust	Stem rust	*barc75*	*Sr24#12*	*iag95*	*csLV34*	*Ventriup+LN2*	*csSr2*
12:ZWW12	KA/NAC//TRCH/3/MUU	*NIL*	*Sr17, Sr30*	-	-	-	-	-	-
13:ZWW12	KA/NAC//TRCH/3/PAURAQ	*Yr?*	*Sr8a, Sr12, Sr17, Sr30+*	-	-	-	-	-	-
15:ZWW12	KA/NAC//TRCH/5/SERI*3//RL6010/4*YR/3/PASTOR/4/BAV92	*NIL*	*Sr30*	-	-	-	-	-	-
16:ZWW12	KA/NAC//TRCH/3/PAURAQ	*Yr27*	*Sr30*	-	-	-	-	-	-
17:ZWW12	KA/NAC//TRCH/3/PAURAQ	*Yr3*	*Sr30*	-	-	-	-	-	-
18:ZWW12	FALCIN/AE.SQUARROSA (312)/3/THB/CEP7780//SHA4/LIRA/4/FRET2/5/ATTILA*2/PBW65	*Yr?*	*Sr17+*	-	-	-	\	-	Null
19:ZWW12	1447/PASTOR//KRICHAUFF/3/PAURAQ	*Yr17, Yr27*	*Sr24, Sr38*	-	+	-	-	+	Null
20:ZWW12	ANNUELLO/3/KA/NAC//TRCH	*Yr3, ?*	*Sr8a*	-	-	-	-	-	-
21:ZWW12	VEE/LIRA//BOW/3/BCN/4/KAUZ/5/DANPHE #1	*Yr3, ?*	*Sr8b, Sr17*	-	-	-	-	-	-
23:ZWW12	WBLL1/PAURAQ	*NIL*	*Sr30*	-	-	-	-	-	-
24:ZWW12	WBLL1/PAURAQ	*NIL*	*Sr30*	-	-	-	+	-	-
25:ZWW12	ASTREB/CHONTE	*NIL*	*Sr8a, Sr17, Sr30*	-	-	-	-	-	-
26:ZWW12	MILAN/KAUZ//DHARWAR DRY/3/BAV92/4/CHONTE	*Yr17*	*Sr12, Sr17, Sr30, Sr38*	-	-	-	\	+	-

QCode	Pedigrees	Genes postulated		*Yr4*	*Lr24/Sr24*	*Lr26/Yr9/Sr31*	*Lr34/Yr18/Sr57*	*Lr37/Yr17/Sr38*	*Sr2*
		Stripe rust	Stem rust	*barc75*	*Sr24#12*	*iag95*	*csLV34*	*Ventriup+LN2*	*csSr2*
28:ZWW12	MILAN/KAUZ//DHARWAR DRY/3/BAV92/4/CHONTE	*Yr17*	*Sr17, Sr30, Sr38*	-	-	-	-	+	-
29:ZWW12	MILAN/KAUZ//DHARWAR DRY/3/BAV92/4/PAURAQ	*Yr6*	*Sr30*	-	-	-	-	-	-
30:ZWW12	MILAN/KAUZ//DHARWAR DRY/3/BAV92/4/PAURAQ	*Yr17*	*Sr8a, Sr30, Sr38*	-	-	-	-	+	+
31:ZWW12	METSO/ER2000/5/2*SERI*3//RL6010/4*YR/3/PASTOR/4/BAV92	*Yr17*	*Sr17, Sr30, Sr38*	-	-	-	-	+	-
32:ZWW12	METSO/ER2000/5/2*SERI*3//RL6010/4*YR/3/PASTOR/4/BAV92	*Yr17*	*Sr8a, Sr17, Sr30, Sr38*	-	-	-	-	+	-
33:ZWW12	AGT YOUNG*2//SUNCO/2*PASTOR	*Yr17*	*Sr24, Sr38*	-	+	-	+	+	Null
34:ZWW12	TOB/ERA//TOB/CNO67/3/PLO/4/VEE#5/5/KAUZ /6/FRET2/7/ATTILA*2/PBW65	*Yr?*	*Sr8b, Sr9b, Sr12+*	-	-	-	+	-	-
35:ZWW12	TOB/ERA//TOB/CNO67/3/PLO/4/VEE#5/5/KAUZ /6/FRET2/7/PASTOR//MILAN/KAUZ/3/BAV92	*Yr3, Yr17*	*Sr8a, Sr17, Sr30, Sr38*	-	-	-	+	+	-
36:ZWW12	TOB/ERA//TOB/CNO67/3/PLO/4/VEE#5/5/KAUZ /6/FRET2/7/PASTOR//MILAN/KAUZ/3/BAV92	*Yr17*	*Sr38+*	-	-	-	+	+	-
37:ZWW12	TOB/ERA//TOB/CNO67/3/PLO/4/VEE#5/5/KAUZ /6/FRET2/7/PASTOR//MILAN/KAUZ/3/BAV92	*Yr6*	*Sr9b*	-	-	-	-	-	-
39:ZWW12	TOB/ERA//TOB/CNO67/3/PLO/4/VEE#5/5/KAUZ /6/FRET2/7/DANPHE #1	*Yr27*	*Sr17, Sr30*	-	-	-	\	-	-
42:ZWW12	WORRAKATTA/2*PASTOR//DANPHE #1	*Yr3, ?*	*Sr24*	-	+	-	\	-	-

QCode	Pedigrees	Genes postulated		*Yr4*	*Lr24/Sr24*	*Lr26/Yr9/Sr31*	*Lr34/Yr18/Sr57*	*Lr37/Yr17/Sr38*	*Sr2*
		Stripe rust	Stem rust	*barc75*	*Sr24#12*	*iag95*	*csLV34*	*Ventriup+LN2*	*csSr2*
43:ZWW12	WORRAKATTA/2*PASTOR//DANPHE #1	*NIL*	*Sr23, Sr30*	-	-	-	-	-	-
44:ZWW12	KRICHAUFF/2*PASTOR//CHONTE	*Yr3, ?*	*Sr24*	-	+	-	-	-	-
45:ZWW12	SUNCO.6/FRAME//PASTOR/3/DANPHE #1	*Yr3, ?*	*Sr30+*	-	-	-	+	-	-
46:ZWW12	KA/NAC//TRCH/3/DANPHE #1	*NIL*	*Sr17, Sr30*	-	-	-	-	-	-
48:ZWW12	KA/NAC//TRCH/3/DANPHE #1	*NIL*	*Sr30*	-	-	-	-	-	-
49:ZWW12	KA/NAC//TRCH/3/DANPHE #1	*NIL*	*Sr30+*	-	-	-	-	-	-
51:ZWW12	BERKUT/MUU//DANPHE #1	*NIL*	*Sr17, Sr8b, Sr9b*	-	-	-	\	-	-
52:ZWW12	AGT YOUNG*2/5/TUI//2*SUNCO/SA1166/3/TUI/4/FINSI	*Yr6*	*Sr8a, Sr17, Sr38*	-	-	-	-	+	Null
53:ZWW12	AGT YOUNG*2/5/TUI//2*SUNCO/SA1166/3/TUI/4/FINSI	*NIL*	*Sr8a, Sr17, Sr38*	-	-	-	+	+	Null
54:ZWW12	AGT YOUNG*2/5/TUI//2*SUNCO/SA1166/3/TUI/4/FINSI	*Yr17*	*Sr8a, Sr17, Sr38*	-	-	-	+	+	Null
55:ZWW12	METSO/ER2000//MONARCA F2007/3/WBLL1*2/KKTS	*Yr17*	*Sr38+*	-	-	-	+	+	-
56:ZWW12	MON/IMU//ALD/PVN/3/BORL95/4/OASIS/2*BORL95/5/EMB16/CBRD//CBRD	*Yr17*	*Sr31, Sr38*	-	+	-	-	+	Null

QCode	Pedigrees	Genes postulated		*Yr4*	*Lr24/Sr24*	*Lr26/Yr9/Sr31*	*Lr34/Yr18/Sr57*	*Lr37/Yr17/Sr38*	*Sr2*
		Stripe rust	Stem rust	*barc75*	*Sr24#12*	*iag95*	*csLV34*	*Ventriup+LN2*	*csSr2*
57:ZWW12	1447/PASTOR//KRICHAUFF/5/2*SERI*3//RL6010/4*YR/3/PASTOR/4/BAV92	*Yr17*	*Sr8a, Sr17, Sr38*	-	-	-	-	+	-
58:ZWW12	1447/PASTOR//KRICHAUFF/5/2*SERI*3//RL6010/4*YR/3/PASTOR/4/BAV92	*Yr17*	*Sr8a, Sr17, Sr38*	-	-	-	+	+	-
59:ZWW12	TUI//2*SUNCO/SA1166/3/TUI/4/FINSI/5/SOKOLL/6/KA/NAC//TRCH	*NIL*	*Sr30*	-	-	-	\	-	-
61:ZWW12	ITP50/3/KA/NAC//TRCH	*Yr?*	*Sr30*	-	-	-	\	-	-
62:ZWW12	EMB16/CBRD//CBRD/3/SUNCO.6/FRAME//PASTOR/4/MILAN/KAUZ//DHARWAR DRY/3/BAV92	*Yr9, Yr17*	*Sr24, Sr31, Sr38*	-	+	+	+	+	-
63:ZWW12	C80.1/3*BATAVIA//2*WBLL1/3/EMB16/CBRD//CBRD/4/MILAN/KAUZ//DHARWAR DRY/3/BAV92	*Yr4, Yr9, Yr17*	*Sr31, Sr38*	+	-	+	\	+	Null
64:ZWW12	C80.1/3*BATAVIA//2*WBLL1/3/EMB16/CBRD//CBRD/4/MILAN/KAUZ//DHARWAR DRY/3/BAV92	*Yr3, Yr9, Yr17*	*Sr31, Sr38*	-	-	+	-	+	Null
67:ZWW12	KA/NAC//TRCH/3/SLVS/ATTILA//WBLL1/4/KA/NAC//TRCH	*NIL*	*Sr30*	-	-	-	-	-	-
68:ZWW12	KA/NAC//TRCH/3/SLVS/ATTILA//WBLL1/4/KA/NAC//TRCH	*NIL*	*Sr8a, Sr30*	-	-	-	-	-	-
69:ZWW12	KA/NAC//TRCH/3/SLVS/ATTILA//WBLL1/4/KA/NAC//TRCH	*NIL*	*Sr8a, Sr30*	-	-	-	-	-	-

QCode	Pedigrees	Genes postulated		*Yr4*	*Lr24/Sr24*	*Lr26/Yr9/Sr31*	*Lr34/Yr18/Sr57*	*Lr37/Yr17/Sr38*	*Sr2*
		Stripe rust	Stem rust	*barc75*	*Sr24#12*	*iag95*	*csLV34*	*Ventriup+LN2*	*csSr2*
70:ZWW12	KA/NAC//TRCH/3/SLVS/ATTILA//WBLL1/4/KA/NAC//TRCH	*NIL*	*Sr8a, Sr30*	-	-	-	-	-	-
77:ZWW12	SUNCO/2*PASTOR/3/SLVS/ATTILA//WBLL1/4/KA/NAC//TRCH	*NIL*	*Sr30*	-	-	-	-	-	-
78:ZWW12	SLVS/3/CROC_1/AE.SQUARROSA (224)//OPATA/5/VEE/LIRA//BOW/3/BCN/4/KAUZ/6/2*KA/NAC//TRCH	*NIL*	*Sr30*	-	-	-	-	-	-
85:ZWW12	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*JANZ/6/SERI*3//RL6010/4*YR/3/PASTOR/4/BAV92/7/VORB	*Yr3, ?, Yr6*	*Sr30*	-	-	-	-	-	-
87:ZWW12	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*JANZ/8/CAL/NH//H567.71/3/SERI/4/CAL/NH//H567.71/5/2*KAUZ/6/WH576/7/WH 542	*Yr27+*	*Sr9b or Sr30*	-	-	-	-	-	-
88:ZWW12	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*JANZ/6/VORB	*Yr27*	*Sr8a,Sr12, Sr30*	-	-	-	+	-	-
97:ZWW12	WORRAKATTA/2*PASTOR//PARUS/PASTOR/3/SOKOLL	*Yr17*	*Sr8a, Sr17, Sr30, Sr38*	-	-	-	+	+	Null
98:ZWW12	TOB/ERA//TOB/CNO67/3/PLO/4/VEE#5/5/KAUZ/6/FRET2/7/VORB	*Yr6*	*Sr8a, Sr30*	-	-	-	+	-	-

QCode	Pedigrees	Genes postulated		*Yr4*	*Lr24/Sr24*	*Lr26/Yr9/Sr31*	*Lr34/Yr18/Sr57*	*Lr37/Yr17/Sr38*	*Sr2*
		Stripe rust	Stem rust	*barc75*	*Sr24#12*	*iag95*	*csLV34*	*Ventriup+LN2*	*csSr2*
100:ZWW12	TOB/ERA//TOB/CNO67/3/PLO/4/VEE#5/5/KAUZ/6/FRET2/7/PASTOR//MILAN/KAUZ/3/BAV92	*Yr17*	*Sr38+*	-	-	-	-	+	-
101:ZWW12	TOB/ERA//TOB/CNO67/3/PLO/4/VEE#5/5/KAUZ/6/FRET2/7/PASTOR//MILAN/KAUZ/3/BAV92	*Yr6*	*Sr8a, Sr12, Sr30*	-	-	-	-	-	-
102:ZWW12	TOB/ERA//TOB/CNO67/3/PLO/4/VEE#5/5/KAUZ/6/FRET2/7/PASTOR//MILAN/KAUZ/3/BAV92	*NIL*	*Sr30+*	-	-	-	-	-	-
107:ZWW12	TOB/ERA//TOB/CNO67/3/PLO/4/VEE#5/5/KAUZ/6/FRET2/7/PAURAQ	*Yr, ?*	*Sr8a, Sr30*	-	-	-	+	-	-
108:ZWW12	NS-732/HER/3/PRL/SARA//TSI/VEE#5/4/FRET2/5/CHONTE	*Yr27*	*Sr8a, Sr30*	-	-	-	-	-	-
109:ZWW12	KA/NAC//TRCH/3/VORB	*Yr6*	*Sr30*	-	-	-	\	-	-
111:ZWW12	KA/NAC//TRCH/4/MILAN/KAUZ//DHARWAR DRY/3/BAV92	*Yr3,?+*	*Sr8b+??, Sr30*	-	-	-	-	-	-
112:ZWW12	KA/NAC//TRCH/3/DANPHE #1	*Yr34,?+*	*Sr8b, Sr12, Sr30*	-	-	-	-	-	-
113:ZWW12	KA/NAC//TRCH/3/DANPHE #1	*Yr3,?*	*Sr8b, Sr12, Sr30*	-	-	-	-	-	-
114:ZWW12	KA/NAC//TRCH/3/DANPHE #1	*Yr34,?*	*Sr8b, Sr12*	-	-	-	-	-	-
116:ZWW12	KA/NAC//TRCH/3/DANPHE #1	*Yr34?*	*Sr8b*	-	-	-	-	-	-
117:ZWW12	KA/NAC//TRCH/3/DANPHE #1	*Yr34, ?*	*Sr8b,Sr9b*	-	-	-	\	-	-

QCode	Pedigrees	Genes postulated		*Yr4*	*Lr24/Sr24*	*Lr26/Yr9/Sr31*	*Lr34/Yr18/Sr57*	*Lr37/Yr17/Sr38*	*Sr2*
		Stripe rust	Stem rust	*barc75*	*Sr24#12*	*iag95*	*csLV34*	*Ventriup+LN2*	*csSr2*
118:ZWW12	KA/NAC//TRCH/3/DANPHE #1	*Yr?*	*Sr30*	-	-	-	-	-	-
119:ZWW12	KA/NAC//TRCH/3/DANPHE #1	*Yr3, ?*	*Sr9b, Sr8b*	-	-	-	-	-	-
120:ZWW12	KA/NAC//TRCH/3/DANPHE #1	*NIL*	*Sr8b, Sr9b, Sr12*	-	-	-	-	-	-
122:ZWW12	KA/NAC//TRCH/3/PAURAQ	*NIL*	*Sr9b*	-	-	-	+	-	-
126:ZWW12	KA/NAC//TRCH/5/SERI*3//RL6010/4*YR/3/PASTOR/4/BAV92	*NIL*	*Sr30*	-	-	-	-	-	-
127:ZWW12	KA/NAC//TRCH/3/KINDE	*Yr17*	*Sr38+*	-	-	-	-	+	-
128:ZWW12	MILAN/KAUZ//DHARWAR DRY/3/BAV92/4/CHONTE	*Yr17*	*Sr38+*	-	-	-	-	+	-
129:ZWW12	MILAN/KAUZ//DHARWAR DRY/3/BAV92/4/CHONTE	*NIL*	*Sr24+*	-	+	-	-	-	-
130:ZWW12	WORRAKATTA/2*PASTOR//MUU/3/DANPHE #1	*Yr17*	*Sr30, Sr38+*	-	-	-	+	+	-
131:ZWW12	MON/IMU//ALD/PVN/3/BORL95/4/OASIS/2*BORL95/5/2*SKAUZ/BAV92	*Yr3, ?*	*Sr8a, Sr17, Sr30*	-	-	-	-	-	+
132:ZWW12	BERKUT/MUU//MUU	*NIL*	*Sr17, Sr30*	-	-	-	+	-	+
134:ZWW12	BERKUT/MUU//DANPHE #1	*NIL*	*Sr17, Sr30 +*	-	+	-	+	-	+
136:ZWW12	EMB16/CBRD//CBRD/4/BETTY/3/CHEN/AE.SQ//2*OPATA	*Yr27*	*Sr17, Sr30*	-	-	-	-	-	-

QCode	Pedigrees	Genes postulated		*Yr4*	*Lr24/Sr24*	*Lr26/Yr9/Sr31*	*Lr34/Yr18/Sr57*	*Lr37/Yr17/Sr38*	*Sr2*
		Stripe rust	Stem rust	*barc75*	*Sr24#12*	*iag95*	*csLV34*	*Ventriup+LN2*	*csSr2*
137:ZWW12	PASTOR//HXL7573/2*BAU*2/3/PFAU/WEAVER//KIRITATI	*NIL*	*Sr17, Sr24*	-	+	-	-	-	-
138:ZWW12	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*JANZ/6/SKAUZ/BAV92	*Yr17*	*Sr8a, Sr9b, Sr17, Sr38*	-	-	-	-	+	-
140:ZWW12	METSO/ER2000/3/PASTOR//HXL7573/2*BAU	*Yr17*	*Sr38+*	-	-	-	-	+	-
141:ZWW12	METSO/ER2000/3/PASTOR//HXL7573/2*BAU	*Yr17*	*Sr38+*	-	-	-	-	+	-
142:ZWW12	METSO/ER2000/3/PASTOR//HXL7573/2*BAU	*Yr17*	*Sr38+*	-	-	-	-	+	-
145:ZWW12	METSO/ER2000//PBW343*2/KUKUNA	*Yr27*	*Sr8a, Sr30*	-	-	-	-	-	Null

‘+’ indicates the cultivar (line) carry the tested genes; ‘-‘indicates the cultivar (line) don't carry the tested genes; ‘?’ presence of unknown resistance genes; \ = missing data

Table 2. List of *P. graminis* f. sp. *tritici* (Pgt) and *P. striiformis* f. sp. *tritici* (Pst) pathotypes used and their virulence spectrum

PBIC	Pathotype	PBIC	Avirulence	Virulence
	Pgt			
103	34-1,2,3,4,5,6,7	74-L-1	*Sr8b,9e,13,24,27,30,32,33,35,37,38,39,40,45,46, Agi,Em,satu*	*Sr5,6,8a,9b,9g,11,12,15,17,36*
205	34-1,2,3,6,7,(8),9	76-L-7	*Sr8b,9e,13,17,24,27,32,33,35,36,37,38,39,40,45,46, Em,satu*	*Sr5,6,8a,9b,9g,11,12,15,30, Agi*
206	34-1,2,3,5,7,8,9	76-L-8	*Sr8a,8b,9e,13,24,27,32,33,35,36,37,38,39,40,45,46, Em,satu*	*Sr5,9b,9g,11,12,15,17,30, Agi*
465	343-1,2,3,5,6,8,9	890005	*Sr8b,9e,9g,13,15,24,27,32,33,35,36,37,38,39,40,45,46, Em,satu*	*Sr5,6,8a,9b,11,12,17, (30), Agi*
279	98-1,2,(3),(5),6	780129	*Sr8b,9e,13,15,24,27,30,32,33,35,36,37,38,39,40,45,46, Agi,Em,satu*	*Sr5,6,8a, (9b),9g,11,12, (17)*
565	34-1,2,7+Sr38	10130	*Sr8a,8b,9b,9e,13,17,24,27,30,32,33,35,36,37,39,40,45,46, Agi,Em,satu*	*Sr5,6,7b,9g,11,15,38*
99	34-2,4,5,7,11	640231	*Sr8a,9b,9e,13,24,27,30,32,33,35,37,38,39,40,45,46, Agi,Em,satu*	*Sr5,6,7b,9g,11,15,17,36*
	Pst			
599	134E16A+*Yr17*+	61639	*Yr1,3,4,5,10,15,24,27,32,33,34,47, SD, Su, ND, Sp*	*Yr2,6,7,8,9,17, A*
617	134E16A+*Yr17*+ *Yr27*+	101975	*Yr1,3,4,5,10,15,24,32,33,34,47, SD, Su, ND, Sp*	*Yr2,6,7,8,9,17, A,27*
444	110E143A+	861725	*Yr1,5,8,9,10,15,17,24,27,32,33,47, Sp,*	*Yr2,3,4,6,7, SD, Su, ND, A,34*
420	108E141A+	831917	*Yr1,5,7,8,9,10,15,17,24,27,32,33,47, Sp*	*Yr2,3,4,6, A, SD, Su, ND,34*
414	104E137A+	821552	*Yr1,5,6,7,8,9,10,27,32,33,47, Sp*	*Yr2,3,4, A,34*

PBIC: Plant Breeding Institute culture number assigned in the cereal rust collection

Greenhouse tests

Experimental materials were sown in 9 cm diameter plastic pots filled with a mixture of pine bark and river sand in a ratio of 2:1. An initial dose of 10 g of water-soluble fertilizer Aquasol® dissolved in 10 litres of tap water was applied to the filled pots before sowing. Four entries were sown per pot as 10 seeds clump. Seven-day old seedlings were fertilized with Urea at the same dose as Aquasol®. Ten to 12-day old seedlings were inoculated with urediniospores of the different Pgt and Pst pathotypes suspended in light mineral oil Isopar-L® using a hydrocarbon pressure pack. Stem rust inoculated seedlings were humidified on water filled steel trays covered with plastic hoods under natural light at 18-20°C for 48 hours, while stripe rust inoculated seedlings were incubated in the dark at 9±2°C for 24 hours. Following incubation, seedlings were moved to microclimate rooms maintained at 25±2°C (stem rust) and at17±2°C (stripe rust). Two sets were planted for screening against Pgt pathotype 34-2,4,5,7,11. One set each was incubated at 25±2°C and 20±2°C post inoculation to enable postulation of temperature-sensitive stem rust resistance genes *Sr6* and *Sr12* (Tsilo *et al.,* 2009). Seedling stem rust assessments were made 14 days after inoculation using the 0-4 scale described in McIntosh *et al.,* (1995), while stripe rust responses were scored 15 days post inoculation using 0-4 scale as described in Bariana and McIntosh, (1993). The description of infection types (ITs) used to classify the reactions to stem rust are: 0 (no visible uredia); (hypersensitive flecks), 1 (small uredia with necrosis), 2 (small to medium sized uredia with green islands surrounded by necrosis and chlorosis), 3 (medium sized uredia with or without chlorosis), 4 (large uredia without chlorosis) and X (heterogenous, similarly distributed all over the leaves). Stripe rust ITs include: 0 (no visible uredia); (necrotic flecks); N (necrotic areas without sporulation), 1 (necrotic and chlorotic areas with restricted sporulation), 2 (moderate sporulation with necrosis and chlorosis), 3 (sporulation with chlorosis) and 4 (abundant sporulation without chlorosis). Infection types 0 to 2 were considered to show avirulence (low infection) for a particular *Sr* gene and infection types 3 to 4 denoted virulence (high infection). Tests were repeated to clarify ambiguous results.

DNA extraction and quantification

Leaf samples of 2 cm length from leaves (2-leaf stage) of each entry were collected in 2 ml tubes and dried on silica gel for 3 days. DNA was extracted from the 95 wheat entries following the procedure described in Bansal *et al.,* (2014). DNA was quantified using a nanodrop ND-100 spectrophotometer and dilutions of 30 ng/µl of genomic DNA were made using deionized water.

Molecular marker genotyping

The entire wheat nursery was genotyped with gene-linked markers to detect the presence of stem resistance genes *Sr2, Sr24, Sr31, Sr38* and *Sr57* and stripe resistance genes *Yr4, Yr9, Yr17* and *Yr18* (Table 3). Hartog (*Sr2*), Janz (*Sr24, Yr18/Sr57*), Sunlin (*Sr26*), AvS/6**Yr9* (*Sr31/Yr9*), Trident (*Sr38/Yr17*) and Rubric (*Yr4*) were included as positive controls for the respective markers.

Table 3. Details of markers used

Gene	Marker	Amplicon (bp)	Forward primer sequence	Reverse primer Sequence	Reference
Yr4	*barc75*	*Yr4Yr4*=132 *yr4yr4*=137 and 139	AGGGTTACAGTTTGCTCTTTTAC	CCCGACGACCTATCTATACTTCTC TA	Somers *et al. (2004)*
Lr24/Sr24	*Sr24#12*	*Sr24Sr24*=500 *sr24sr24*=null	CAC CCG TGA CAT GCT CGT A	AAC AGG AAA TGA GCA ACG ATG T	Mago *et al. (2005)*
Lr26/Yr9/Sr31	*iag95*	*Yr9Yr9*=1100 *yr9yr9*=null	CTCTGTGGATAGTTACTTGATCG A	CCTAGAACATGCATGGCTGTTACA	Mago et al. (2007)
Lr34/Yr18	*csLV34*	*Lr34Lr34*=150 *lr34lr34*=229	GTTGGTTAAGACTGGTGGTGAT GG	TGCTTGCTATTGCTGCTGAATAGT	Lagudah et al. (2007)
Lr37/Yr17/Sr38	*Ventriup+LN2*	*Yr17Yr17*=262 *yr17yr17*=null	AGGGGCTACTGACCAAGGCT	TGCAGCTACAGCAGTATGTACACA AAA	Helguera et al. (2007)
Sr2	*csSr2*	*Sr2Sr2*=337 *sr2sr2*=null	CAA GGG TTG CTA GGA TTG GAA AAC	AGA TAA CTC TTA TGA TCT TAC ATT TTT CTG	Mago *et al. (2010)*

PCR amplifications were performed at the University of Sydney Plant Breeding Institute, Cobbiity, Australia in 10 µl reaction volumes containing 60 ng/µl of genomic DNA from each entry and respective controls, 0.2 mM dNTPs, 1× PCR buffer containing 1.5 mM $MgCl_2$ (Bioline), 0.5 µM of each primer (forward and reverse) and 0.02 U Immolase Taq DNA polymerase (Bioline). PCR reactions were performed in T100™ thermal cycler (BioRad USA) using published PCR conditions/profiles for the different primers. PCR products and restriction enzyme digests (*csSr2*) were separated on 2% agarose gels stained with gel red and visualised under UV gel documentation system (UVP-GelDoc-It). PCR amplification for M_{13}-labelled *barc75* was carried out in a total reaction volume of 10 µl containing 30 ng/µl of genomic DNA, 1×$MgCl_2$ buffer, 0.75× dNTPs, 0.4×1.25 µM forward primer labelled with M_{13}, 0.4×5 µM Reverse primer, 0.1×0.50 µM M_{13}-tailed primer labelled with IRDye 700 or 800 and 0.04 µl×0.02 U Immolase Taq DNA polymerase (Bioline). The PCR reactions were carried out in BioRad machine and PCR products were separated on 6.5% Polyacrylamide gel using electrophoresis apparatus LICOR-4300 DNA analyser system (Li-COR Bio-science USA). GeneRuler™ 1Kb ladder (Fermentas) was used to determine allele sizes.

Results

Postulation of resistance genes

Resistance genes were postulated by comparing infection types (ITs) of the different Pgt and Pst pathotypes on test genotypes with those of differential lines with known resistance genes. A high IT (3-4) on a test genotype demonstrated the lack of resistance gene for which that pathotype was avirulent and the differential stock for the target gene also expressed high IT. Genotypes that show low ITs (0-2) with all pathotypes are likely to carry either a gene effective against all pathotypes or combinations of genes with compensating pathotypic specificities. Fig. 1 illustrates the various rust responses observed among the wheat screening nursery tested.

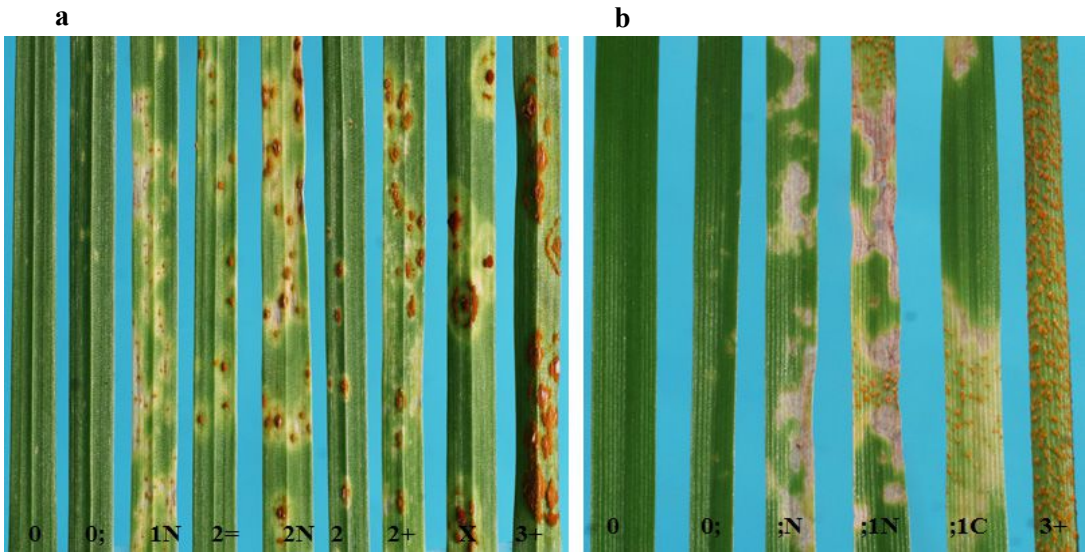

Fig. 1 a) Seedling stem rust **b**) seedling stripe rust response variations observed among the 95 entries tested in this international wheat nursery.

Stem rust

The performance of the 95 wheat lines tested against the seven pathotypes is summarized in Table 4. Seventy-three (76.8%) of the tested entries showed varying levels of resistance (ITs 0, ;, ;1, 1C, and 2) to races 34-1,2,3,4,5,6,7 (103); 34-1,2,3,6,7,(8),9 (205); 34-1,2,3,5,7,8,9 (206); 343-1,2,3,5,6,(8),9 (465); 98-1,2,(3),(5),6 (279); 34-1,2,7+*Sr38* (565); 34-2,4,5,7,11 (99). The remaining 23.2% showed varying levels of susceptibility (ITs 3, 3-, 3+, and 4) Seedling stem rust resistance genes *Sr8a, Sr8b, Sr9b, Sr12, Sr17, Sr23, Sr24, Sr30, Sr31* and *Sr38* were postulated (Table 1 and Table 5). Majority of these genes were present in combinations and a few lines carried *Sr8a* (1), *Sr8b* (1), *Sr9b* (2), *Sr24* (2) and *Sr30* (17) singly. Thirty entries carried *Sr8a* in combinations with one to three genes including *Sr9b*, *Sr12*, *Sr17*, *Sr23*, *Sr30* and *Sr38*. Similarly, *Sr8b* was postulated in nine entries in combination with *Sr9b*, *Sr12*, *Sr17* and *Sr30*. *Sr30*, *Sr38*, *Sr17* and *Sr8a* were among the most predominant stem rust resistances genes detected in this nursery. *Sr24* was postulated in seven entries and *Sr31* in four lines.

Table 4. Proportion of resistant and susceptible entries when tested against *P. graminis* f. sp. *tritici* pathotypes

Pgt Race	Resistant		Susceptible	
	Number of entries	Percentage	Number of entries	Percentage
34-1,2,3,4,5,6,7 (103)	91	95.8	4	4.2
34-1,2,3,6,7, (8),9 (205)	67	70.5	28	29.5
34-1,2,3,5,7,8,9 (206)	69	72.6	26	27.4
343-1,2,3,5,6, (8),9 (465)	51	53.7	44	46.3
98-1,2, (3), (5),6 (279)	80	84.2	15	15.8
34-1,2,7+Sr38 (565) Hot	91	95.8	4	4.2
34-1,2,7+Sr38 (565) Cold	92	96.8	3	3.2
34-2,4,5,7,11 (99) Hot	91	95.8	4	4.2
34-2,4,5,7,11 (99) Cold	93	97.9	2	2.1
All tested races	73	76.8	22	23.2

Table 5. Proportion of resistant and susceptible wheat entries when tested against five *P. striformis* f. sp. *tritici* pathotypes

Pst Race	Resistant		Susceptible	
	Number of entries	Percentage	Number of entries	Percentage
134 E16A+*Yr17*+ (599)	54	56.8	41	43.2
134 E16A+*Yr17*+*Yr27* (617)	39	41.1	56	58.9
110 E143A+ (444)	54	56.8	41	43.2
108 E141A+ (420)	52	54.7	43	45.3
104 E137A+ (414)	33	34.7	62	65.3
All tested races	8	8.4	87	91.6

Table 6. Seedling stem rust resistance variation in the CIMMYT international wheat screening nursery

Resistance genes detected	Frequency	Entries
Sr8a	1	(20:ZWW12)
Sr8a, Sr9b, Sr17, Sr38	1	(138:ZWW12)
Sr8a, Sr9g, Sr12, Sr17, Sr30, Sr38	1	(11:ZWW12)
Sr8a, Sr12, Sr17, Sr30, unknown	1	(13:ZWW12)
Sr8a, Sr12, Sr30	2	(88:ZWW12, 101:ZWW12)
Sr8a, Sr12, Sr17, Sr30	1	(1:ZWW12)
Sr8a, Sr17, Sr30, Sr38	5	(8:ZWW12, 10:ZWW12, 32:ZWW12, 35:ZWW12, 97:ZWW12)
Sr8a, Sr17, Sr30	4	(2:ZWW12, 3:ZWW12, 25:ZWW12, 131:ZWW12)
Sr8a, Sr17, Sr38	5	(52:ZWW12, 53:ZWW12, 54:ZWW12, 57:ZWW12, 58:ZWW12)
Sr8a, Sr23, Sr30	1	(4:ZWW12)
Sr8a, Sr30, Sr38	1	(30:ZWW12)
Sr8a, Sr30	7	(68:ZWW12, 69:ZWW12, 70:ZWW12, 98:ZWW12, 107:ZWW12, 108:ZWW12, 145:ZWW12)
Sr8b	1	(116:ZWW12)
Sr8b, Sr9b	2	(117:ZWW12, 119:ZWW12)
Sr8b, Sr9b, Sr12	1	(120:ZWW12)
Sr8b, Sr9b, Sr12+	1	(34:ZWW12)
Sr8b, Sr9b, Sr17	1	(51:ZWW12)
Sr8b, Sr12, Sr30	2	(112:ZWW12, 113:ZWW12)
Sr8b, Sr12	1	(114:ZWW12)
Sr8b, Sr17	1	(21:ZWW12)
Sr8b, Sr30, unknown	1	(111:ZWW12)
Sr9b	2	(37:ZWW12, 122:ZWW12)
Sr9b, Sr30	1	(87:ZWW12)
Sr12, Sr17, Sr30, Sr38	1	(26:ZWW12)
Sr12, Sr36, Sr38	1	(9:ZWW12)
Sr17, Sr24	1	(137:ZWW12)
Sr17, Sr30	5	(12:ZWW12, 39:ZWW12, 46:ZWW12, 132:ZWW12, 136:ZWW12)
Sr17, Sr30, unknown	1	(134:ZWW12)
Sr17, Sr30, Sr38	2	(28:ZWW12, 31:ZWW12)
Sr17+	1	(18:ZWW12)
Sr23, Sr30	1	(43:ZWW12)
Sr24	2	(42:ZWW12, 44:ZWW12)
Sr24+	1	(129:ZWW12)
Sr24, Sr31, Sr38	1	(62:ZWW12)
Sr24, Sr38	2	(19:ZWW12, 33ZWW12)
Sr26, Sr38	1	(55:ZWW12)
Sr30	17	(5:ZWW12, 15:ZWW12, 16:ZWW12, 17:ZWW12, 23:ZWW12, 24:ZWW12, 29:ZWW12, 48:ZWW12, 59:ZWW12, 61:ZWW12, 67:ZWW12, 77:ZWW12, 78:ZWW12, 85:ZWW12, 109:ZWW12, 118:ZWW12, 126:ZWW12)
Sr30, unknown	3	(45:ZWW12, 49:ZWW12, 102:ZWW12)
Sr30, Sr38, unknown	1	(130:ZWW12)

Resistance genes detected	Frequency	Entries
Sr31, Sr38	3	(56:ZWW12, 63:ZWW12, 64:ZWW12)
Sr38, unknown	7	(36:ZWW12, 100:ZWW12, 127:ZWW12, 128:ZWW12, 140:ZWW12, 141:ZWW12, 14:ZWW12)

Table 7. Seedling stripe rust resistance variation in the CIMMYT international wheat screening nursery

Resistance genes postulated	**Frequency**	**Entry**
Yr3	11	(1:ZWW12, 2:ZWW12, 17:ZWW12, 20:ZWW12, 21:ZWW12, 42:ZWW12, 44:ZWW12, 45:ZWW12, 113:ZWW12, 119:ZWW12, 131:ZWW12)
Yr3, unknown	2	(35:ZWW12, 111:ZWW12)
Yr3, Yr6	1	(85:ZWW12)
Yr3, Yr9, Yr17	1	(64:ZWW12)
Yr4, Yr9, Yr17	1	(63:ZWW12)
Yr6	6	(29:ZWW12, 37:ZWW12, 52:ZWW12, 98:ZWW12, 101:ZWW12, 109:ZWW12,)
Yr9, unknown	1	(18:ZWW12)
Yr9, Yr17	1	(62:ZWW12)
Yr17	24	(9:ZWW12, 10:ZWW12, 11:ZWW12, 26:ZWW12, 28:ZWW12, 30:ZWW12, 31:ZWW12, 32:ZWW12, 33:ZWW12, 36:ZWW12, 54:ZWW12, 55:ZWW12, 56:ZWW12, 57:ZWW12, 58:ZWW12, 97:ZWW12, 100:ZWW12, 127:ZWW12, 128:ZWW12, 130:ZWW12, 138:ZWW12, 140:ZWW12, 141:ZWW12, 142:ZWW12)
Yr17, Yr27	2	(8:ZWW12, 19:ZWW12)
Yr27	8	(4:ZWW12, 5:ZWW12, 16:ZWW12,39:ZWW12, 88:ZWW12, 108:ZWW12, 136:ZWW12, 145:ZWW12)
Yr27, unknown	1	(87:ZWW12)
Yr34	3	(114:ZWW12, 116:ZWW12, 117:ZWW12)
Yr34, unknown	1	(112:ZWW12)
Unknown	7	(3:ZWW12, 13:ZWW12, 18:ZWW12, 34:ZWW12, 61:ZWW12, 107:ZWW12, 118:ZWW12)
None	26	(12:ZWW12, 15:ZWW12, 23:ZWW12, 24:ZWW12, 25:ZWW12, 43:ZWW12, 46:ZWW12, 48:ZWW12, 49:ZWW12, 51:ZWW12, 53:ZWW12, 59:ZWW12, 67:ZWW12, 68:ZWW12, 69:ZWW12, 70:ZWW12, 77:ZWW12, 78:ZWW12, 102:ZWW12, 120:ZWW12, 122:ZWW12, 126:ZWW12, 129:ZWW12, 132:ZWW12, 134:ZWW12, 137:ZWW12)

Stripe rust

Eight (8.4%) of the 95 tested wheat entries showed different resistance levels to the five races of stripe rust at the seedling stage (Table 5). The rest of 87 (91.6%) wheat entries showed varying

levels of susceptibility. Stripe rust multi-pathotype testing results are summarized in Table 1 and 7. Screening with five different Pst pathotypes detected seven seedling stripe rust resistance genes (*Yr3, Yr4, Yr6, Yr9, Yr17, Yr27* and *Yr34*) either singly or in combinations. *Yr3* was present singly in 11 entries and in combination with *Yr6* in one line (85:ZWW12). *Yr3* in combination with an additional gene was postulated in entries 35:ZWW12 and 111:ZWW12. *Yr3* in combination with *Yr9* and *Yr17* was detected in entry 64:ZWW12. Stripe rust resistance gene *Yr4* in combination with *Yr9* and *Yr17* was postulated in entry 63:ZWW12. *Yr6* was detected singly in six entries (Table 1, 7). *Yr9* and *Yr17* were postulated in one line (62:ZWW12). Additionally, *Yr17* was present singly in 24 entries and in combinations with *Yr27* in two lines. Likewise, *Yr27* was detected singly in eight entries and in combination with additional unknown resistance in one line (87:ZWW12). On the other hand, *Yr34* was postulated singly in three lines and in combination with extra unknown resistance in one entry. Seven entries carried resistance genes that could not be detected by the array of Pst pathotypes used. No seedling stripe rust resistance genes were postulated in 26 entries. ASR genes *Yr17* (32%) followed by *Yr3* (16%) and *Yr27* (12%) were the most frequent resistance genes detected. *Yr4* (1%), *Yr9* (3%) and *Yr34* (4%) were the least frequently detected.

Molecular marker genotyping

The marker *csSr2,* detected APR gene *Sr2* in four entries (Hope allele; 172bp, 112bp and 53bp), whereas 80 entries amplified the Marquis type allele (225bp and 112bp) after digestion with the restriction enzyme *Bsp*HI. Eleven entries did not amply any product. Out of the 95 entries genotyped with the dominant STS marker *Sr24#12*, only nine entries and the positive control Janz produced a 500 bp amplicon associated with *Sr24*, whereas no amplification was observed in the remaining 86 entries. The marker *iag95* detected *Sr31/Yr9/Lr26* in three entries, while the marker *Ventriup + LN2* confirmed the presence of *Sr38/Yr17/Lr37* in 32 entries. One genotype was confirmed to carry *Yr4* when genotyped with SSR marker *barc75*. Based on marker *csLV34*, 22 entries were observed to carry the pleiotropic APR gene *Yr18/Lr34/Sr57* (Table 1). Of the 22 entries confirmed to carry *Yr18*, three entries (24:ZWW12, 53:ZWW12 and 132:ZWW12) were susceptible at the seedling stage.

Discussion

Successful deployment of rust resistance genes depends on a better understanding of the genetic diversity among donor sources (Bariana *et al.,* 2007a). The main objective of this study was to assess the genetic diversity for stem rust and stripe rust resistance in an international wheat screening nursery. Strategic deployment of stem rust resistance started in Australia with the release of cultivars Hofed and Fedweb in 1937 (Macindoe and Walkden-Brown, 1968). The *Sr11* carrying cultivar Gabo was released in 1945 and became backbone of the CIMMYT's wheat improvement program due to it photoperiod insensitivity.

Multi-pathotype evaluations identified stem rust ASR genes *Sr8a, Sr8b, Sr9b, Sr12, Sr17, Sr23, Sr24, Sr30, Sr31* and *Sr38* and stripe rust resistance genes *Yr3, Yr4, Yr6, Yr9, Yr17, Yr27* and *Yr34* either singly or in combinations. Unfortunately, most of these genes are not effective individually against at least one of the Pgt and Pst pathotypes worldwide (Singh *et al.,* 2008b). Postulation of the above-mentioned stem rust and stripe rust seedling resistance genes is expected because these seem to be fixed in breeding populations due to their widespread use. For example; Singh *et al.,* (2008a) postulated eight stem rust resistance genes (*Sr5, Sr8a, Sr9g,*

Sr12, Sr30, Sr31, Sr36 and *Sr38*) and seven stripe rust resistance genes (*Yr1, Yr6, Yr7, Yr9, Yr17, Yr27*, *YrHVII*) either singly or in combinations in wheat cultivars from the United Kingdom. Admassu *et al.,* (2012), reported 11 stem rust resistance genes (*Sr5, Sr7a, Sr7b, Sr8a, Sr9e, Sr11, Sr21, Sr27, Sr29, Sr30* and *Sr37*) either singly or in combinations in durum and bread wheat cultivars and breeding lines from Ethiopia. Spanic *et al.,* (2015) reported four stem rust resistance genes (*Sr8a, Sr31, Sr36, Sr38*) in Croatian wheat cultivars. Kolmer *et al.,* (2007) when comparing the frequency of stem rust resistance genes in United States winter and spring wheats found that resistance genes *Sr2, Sr6, Sr17, Sr24, Sr31, Sr36* and *SrTmp* were common in winter wheats, while genes *Sr6, Sr9b, Sr11* and *Sr17* were more frequent in spring wheats. This study found *Sr8a, Sr12, Sr17, Sr30* and *Sr38* to be more common in CIMMYT spring wheat nursery. Postulation of common genes in different studies is attributed to the use of CIMMYT germplasm directly or as parents in many countries (Ortiz *et al.,* 2007; 2008; Pretorius *et al.,* 2015). It is estimated that about 70-80 % of spring wheat cultivars released in the developing world are CIMMYT lines or lines derived from CIMMYT parents (Wang *et al.,* 2003; Ortiz *et al.,* 2007) indicating the level of international effort to breed against deadly pathogens and wider adaptation, and the importance of CGIAR centres like CIMMYT in providing improved germplasm to national breeding programs in developing countries.

Sr30 was the most frequent stem rust seedling resistance gene. Although a Pgt pathotype virulent on *Sr30* was reported in Eastern Australia by Park and Wellings, (1992), it is still effective against commercially important pathotypes (Bariana *et al.,* 2007b). *Sr30* virulence was also reported in many other countries including Spain, Ethiopia, Turkey, Pakistan and South American countries (Huerta-Espino, 1992). *Sr30* is the backbone of the CIMMYT and Australian germplasm (McIntosh *et al.,* 1995, Bariana *et al.,* 2007). Other stem rust resistance genes detected in high frequency were *Sr38* (34%), *Sr8a* (33%), *Sr17* (33%) and *Sr12* (13%). Virulence for *Sr38* was first detected in Western Australia in 2001 (Park, 2008); however, this gene is still being used in breeding programs because of its linkage with cereal cyst nematode gene *Cre5* (Jahier *et al.,* 2001). Resistance genes *Sr31, Sr24* and *Sr23* were detected at a very low frequency in this nursery. The virulence in Ug99 and its variants on these genes has possibly been responsible for this trend (Singh *et al.,* 2015).

It is surprising not to detect stem rust resistance genes *Sr13*, *Sr15* and *Sr36* in this study. *Sr13* has not been deployed widely, except in some Australian cultivars such as, Miskle and Machete (H.S. Bariana unpublished results). The incorporation of *Sr15* and *Sr36* in CIMMYT's wheat improvement program is likely to happen due to association of genomic regions carrying these genes with root lesion nematode resistance gene *Rlnn1* (Jayatilake *et al.,* 2013) and crown rot resistance respectively. Stem rust resistance genes *Sr33* and *Sr45* were deployed in Australian wheat cultivars Lorikeet and Thornbill, respectively (H.S. Bariana unpublished results). Overall, the cultivars identified with known stem rust resistance genes will provide valuable genetic material for breeding resistant wheat cultivars.

The most predominant seedling stripe rust resistance genes detected were *Yr17* (34%), *Yr3* (16%) and *Yr27* (12%). Pathan, (2003) reported the presence of *Yr17/Lr37/Sr38* (VPM) cluster in many European wheats. The VPM segment has been widely deployed in commercial cultivars in many parts of the world including Australia (Park, 2008). The popularity of this useful translocation has declined due to reported virulences for all the three rust resistance genes (Singh *et al.,* 2008a). Virulence for *Yr17* was first detected in eastern Australia in 1999 and was thought to have originated from an existing pathotype via mutation (Wellings, 2007) and by 2006 a pathotype with combined virulence for *Yr17*, *Yr6*, *Yr7* and *YrA* was identified (Wellings, 2007).

The second highly frequent stripe rust resistance gene in this study was *Yr3* (16%). *Yr3* was also postulated in CIMMYT wheat germplasm by Dubin *et al.,* (1989). *Yr3* was not an important gene for Australia until the detection of WA pathotype in 2002, which carried virulence for *Yr6, Yr7, Yr8, Yr9,* and *YrA* and avirulence for *Yr3* and *Yr4* (Wellings *et al.,* 2003; Wellings and Kandel, 2004). The effectiveness of the 1BL.1RS (*Lr26/Yr9/Sr31*) translocation in protecting wheat against stem rust for over 30 years before the detection of Ug99 in 1998 (Pretorius *et al.,* 2000), led to high frequency of these three genes in most wheats globally (Singh *et al.,* 2008b). High proportions of *Yr9* have been reported in Chinese wheat cultivars (Zeng *et al.,* 2014). Pathan *et al.,* (2008) also reported a high frequency of *Yr9* in European wheats. Singh *et al.,* (2014) postulated the stripe rust resistance gene *Yr9* (1BL.1RS rye-derived) in 58% of the 12th High Temperature Wheat Yield Trial (12th HTWYT), 17% of the 22nd Semi-Arid Wheat Screening Nursery (22nd SAWSN) and 2% entries of the 1st Australian Special Nursery (1st ASN). On the contrary, the *Yr9*-rye translocation was detected in only 3% of the entries in the nursery screened in the present study. The declining frequency of *Yr9* in CIMMYT germplasm could be due to the reported virulence for *Sr31* and *Yr9* located on the IBL-1RS translocation (Pretorius *et al.,* 2000; Wellings *et al.,* 2003). Seedling stripe rust resistance gene *Yr27* was present in a number of CIMMYT wheats including Ciano 79, Nacozari 76, Crow, Tesia 79, Opata 85, Bacanora 88, Bakhtawar, WH542, Atrak, Memof, PBW343, MH97, Chamaran, Kubsa, and Shirudi (Wellings, 1992). However, the outbreak of *Yr27* virulent pathotype in 2010-2013 caused significant yield losses in Afghanistan, Azerbaijan, Ethiopia, Iran, Iraq, Kenya, Morocco, Syria, Turkey and Uzbekistan (Singh *et al.,* 2012; FAO, 2014). The ineffectiveness of this widely deployed resistance gene posed a serious threat to food security and livelihoods of resource-poor farmers and their communities.

Yr34 was mapped on chromosome 5AL of wheat genotype WAWHT2046 (Bariana *et al.,* 2006). It is effective against 134 E16A+ and its variants. It was only present in 4% of the entries. Seven entries carried resistance that could not be postulated by the array of Pst pathotypes used in this study indicating that they carry either new (uncharacterized) genes effective against all pathotypes used in this study or combinations of genes. Twenty seven percent of entries were susceptible at the seedling stage to all Pst pathotypes used and field testing is recommended to determine their field response to stripe rust at the adult plant stage.

This investigation explained the diversity in CIMMYT wheat germplasm through postulation of known genes for resistance to stem rust and stripe rust diseases using phenotypic assessments against several pathotypes of each rust pathogen and markers linked with APR genes. Genotypes carrying potentially new uncharacterized ASR genes for stem rust and stripe rust resistance against Australian Pst pathotypes were identified for formal genetic analysis. The genotypes with good level of resistance to the two rusts have been identified for incorporation into breeding programs as donor parents. The information presented in this study is useful for wheat breeders to devise strategies for achieving durable rust control.

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