



# Characterization of exotic germplasm lines for resistance to wheat rusts and spot blotch

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## Abstract

Breeding resistant varieties of wheat is a continuous process and empirical disease control depends on deployment of diverse sources of genetic resistance to the deadly rusts. A total of 166 exotic wheat germplasm accessions were evaluated for resistance to wheat rusts having some component of resistance to either any of the three rusts and spot blotch. The data for adult plant resistance (APR) on leaf and stem rusts, was recorded at Wellington while yellow rust epiphytotic was artificially created at Karnal for identification of resistant lines. Based on APR data 85, 96 and 16 accessions were found completely resistant to leaf, stem and stripe rusts respectively. Based on the seedling resistance data against different pathotypes (10 each for leaf and stripe and 5 for stem rust) of each rust, nine, fifteen and five accessions were found highly resistant respectively. Based on the multi-environment adult plant resistance data and race specific seedling data, we were able to identify 28, accessions highly resistant to either one or two rusts. For spot blotch the evaluation was done at hotspot locations Wadura and Cooch-behar and 26 accessions were found to be highly resistant against this disease. The lines so identified in this study are being used in the hybridization programs to enhance the levels of resistance.

**Keywords** Wheat rusts · Seedling resistance test · Adult plant resistance · Spot blotch

## Introduction

The target population to feed by 2050 will be an additional 2.3 billion people and for that to happen we need to produce 70% more food (Alexandratos and Bruinsma 2012; Tilman

et al. 2011). To satisfy the hunger of escalating population, unhindered production of wheat is the most challenging task towards world agriculture. In this context sustainable production of wheat to reach the estimated goals is of supreme importance. Wheat production, however, is impeded by various biotic and abiotic factors that affect the yields creating hurdles to achieve the targeted objectives. Among the biotic constraints, prevalence of different rust pathogens is of primary importance. Rust disease cause substantial economic losses, damaging the crop everywhere in the world (Huerta-Espino et al. 2011). Globally three wheat rusts viz. yellow rust (*Puccinia striiformis* f. sp. *tritici*), stem rust (*Puccinia graminis* f. sp. *tritici*) and leaf rust (*Puccinia triticina*) and are the most damaging diseases of wheat (Roelfs et al. 1992).

Wheat rusts are known to cause extensive yield loss depending on environmental conditions prevailing in particular area, which may lead to disease epidemics (Pardey et al. 2013; Singh et al. 2016). Leaf rust is the most prevalent of the three rusts. Leaf rust is of major economic importance worldwide causing considerable yield losses under large geographical regions (Roelfs et al. 1992; Singh et al. 2014). Yield losses caused due to leaf rust are variable, due

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to differences in climatic conditions, variety susceptibility and presence of inoculums. Yield losses caused by leaf rust are due to infection of the flag leaf that has crucial role to play in grain filling. Grain losses of up to 30–70% can be inflicted by leaf rust if susceptible cultivars are grown (Mur-ray et al. 1994; Ordonez et al. 2010).

*Puccinia graminis* f. sp. *tritici* (Pgt), the casual fungus of wheat stem rust is the most damaging rust of wheat. Stem rust epidemics have been prevalent in the wheat growing areas across the world. Resistant cultivars are continuously being developed to avoid such epidemics. Resistance however can be broken by new pathogenic variability. Physiologic races are known to exist in *P. graminis* f. sp. *tritici* (Roelfs et al. 1987). Ug99 identified in Uganda in 1998 was found virulent to many stem rust resistance genes including the widely grown *Sr31* (Jin 2005). Stem rust of wheat became a significant danger to the wheat production after the emergence of this pathotype Ug99 (TTKSK) (Pretorius et al. 2000). In stem rust infected plants nutrient flow to developing ears is restricted which results in grain shriveling and thus yield loss (Roelfs et al. 1992).

Yellow rust of wheat is a highly widespread disease and is prevalent across the major wheat growing regions in the world (Stubbs 1985; Manners 1988; Singh et al. 2004). Beddow et al. (2015) estimated that the global yield losses of approximately 5.5 million tons per year are due to stripe rust of wheat. Since the beginning of this century, localized yellow rust epidemics have been reported from many wheat growing regions, including Africa and Central Asia (Ezzahiri et al. 2009; Rahmatov et al. 2012; Singh et al. 2016). Yellow rust incidences in recent years have increased (Hovmøller et al. 2007) with high frequency (Wellings 2007; Hovmøller et al. 2016). This is mainly attributed to high rates of transformations in the pathogen (Hovmøller and Justesen 2007), long distance dispersal (Zadoks 1961; Brown and Hovmøller 2002) and emerging new races (Rodriguez-Algaba et al. 2014). With evolution of more virulent pathotypes the primitive races are being replaced by new lineages which have led to high rates of epidemics on in recent years (Rahmatov et al. 2017).

Spot blotch caused by *Bipolaris sorokiniana* is attaining serious concern in South Asia including eastern parts of India as this disease affects grain size, yield and quality. Under favourable conditions, spikelets may be affected, causing grain shrivelling (Kumar et al. 2002). The average yield losses due to spot blotch are reported ranging 18–50% under disease favourable conditions (Singh et al. 2015).

Wheat breeders generally deploy two classes of resistant sources in the breeding programs. Seedling resistance (all stage resistance) and adult plant resistance genes have been documented to confer resistance at different plant growth stages. The present study plans to characterize a set of exotic germplasm lines received from ICARDA to identify

potential new sources of resistance to wheat rusts and blotch diseases.

## Materials and methods

### Experimental material

The experimental material for conducting this study was procured from International Center for Agricultural Research on Dryland Areas (ICARDA) through the National Genebank at ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR) New Delhi. 166 accessions were obtained and studied exhaustively. Most of these accessions had resistance to either any of the three wheat rusts and spot blotch. These lines were tested for resistance to three rusts and spot blotch during 2014–2015 and 2015–2016 crop seasons. Each accession was sown in single row (1 m length). The distance between two successive rows was kept 25 cm. Border rows of each block (each block contained 20 genotypes) were planted with infector genotypes for proper disease screening.

### Screening for resistance to wheat rusts

All the 166 wheat accessions were evaluated at both seedling and adult plant stage to identify potential new rust resistance sources. For seedling stage resistance analysis accessions were evaluated against virulent and predominant pathotypes of all the three wheat rusts at Regional Station, Shimla ICAR-IIWBR. Evaluation for identification of adult plant resistances was carried out at Wellington, Tamil Nadu for black and Leaf rusts and at Karnal for stripe rust. The stripe rust epiphytotic was created artificially under field conditions at Karnal.

### Evaluation for rust resistance at seedling stage

For seedling stage resistance evaluation, seedlings were planted in aluminum trays containing autoclaved mixture of fine loam and manure in 3:1 ratio. About 5–6 seeds were sown for each genotype. Along with the tested genotypes known gene lines were also evaluated for seedling stage resistance evaluation. Spore-proof greenhouse chambers maintained at  $22 \pm 2$  °C, 50–70% relative humidity and 12-h daylight were used to raise the seedlings. One week old seedlings were inoculated. The concentration of spores was maintained at 10 mg of spores of each genotype suspended in 2 ML light grade mineral oil. The suspension containing spores in mineral oil was evaporated for 30 min followed water misting. Treated plants were placed in dew chambers for 24 h at  $16 \pm 2$  °C and  $20 \pm 2$  °C temperature, 100% relative humidity and 12 h' daylight for yellow and brown rust, respectively. Plants were transferred to a greenhouse

maintained at  $16 \pm 2$  °C and  $20 \pm 2$  °C temperature, relative humidity of 40–60%, and illuminated at about 15,000 lx for 12 h for yellow and brown rust, respectively. Infection types (resistant or susceptible) on the test lines were observed 14 days after inoculation according to Stakman et al. (1962) method.

**Rust resistance gene postulation using multi-pathotype data**

Evaluation of rust genes in wheat lines was inferred using the gene-matching technique using multi-pathotype data (Browder 1973). During resistance gene(s) postulation additional information like genetic linkage between the different resistance genes, pedigree of the wheat lines/varieties, and morphological markers were also considered.

**Inferring adult plants resistance to rusts** Wellington (Tamil Nadu) is the most preferred location for screening of brown and black rusts (Nagarajan and Joshi 1985), as the environment required for the spread of disease prevails naturally in this location. Each accession was planted and evaluated separately for adult plant resistance to stem and leaf rusts at Wellington during December 2014–April 2015. For the purpose of comparison of results Agra Local was used as the susceptible check and HW5216 as resistant check. For screening of adult plant resistance to yellow rust, epiphytotic was created artificially under field conditions at Karnal. For each genotype, 10 individual plants were scored. Rust response of individual accessions was recorded after completion of Zadok 70 stage (Zadoks et al. 1974). Data was recorded using modified Cobb scale (Peterson et al. 1948).

**Spot blotch resistance evaluation** The screening for spot blotch was done at Coochbehar under natural conditions. To ensure uniform spreading of disease, border rows after each block (20 genotypes) were planted along with the genotypes. Double digit (DD) score was recorded based on *per cent* leaf area covered by the disease on the flag and the

penultimate leaf. The data was recorded at three stages. The disease severity (DS) was calculated as given below;

$$Disease\ Severity\% = (D1/9) \times (D2/9) \times 100$$

**Results and discussion**

Firstly, we present the multiple race-based resistance identified at seedling stage for all the three rusts. Also, the genes postulated based on seedling reactions are discussed in the light of their effectiveness. Secondly, we discuss the resistance identified in the set of lines based on field screening for three rusts. In order to identify potential rust resistance sources, the stem and leaf rust data from wellington and stripe rust data from Karnal was used. Finally, the data for spot blotch on this set from Wadura and Coochhehar locations are discussed.

**SRT (seedling resistance test) based identification of rust resistance lines**

Based on the SRT data against different pathotypes of leaf rust fungus *P. triticina*, nine accessions (6, 7, 36, 142, 143, 144, 145, 146, 147) were found highly resistant against ten pathotypes (12-2, 12-5, 77-2, 77-5, 77A-1, 104-B, 104-2, 77-2, 77-8, 77-10) of leaf rust. Accessions conferring high resistance against all the tested pathotypes are given in Table 1. All the 9 accessions found resistant in seedling stage were also resistant at APR stage (all showed immune reaction in APR) and most of them have *Lr26* gene either alone or in combination with other *Lr* genes. So, it may be concluded that the resistance conferred by these accessions may be due to combinatorial presence of *Lr26* with other genes.

The 166 accessions were tested for resistance at seedling stage against five fungal pathotypes viz. 11, 21A-2, 24A, 40-2 and 117-3. Accessions that were recorded resistant for most of the pathotypes are given in Table 2. Overall only five

**Table 1** Accessions showing resistance reaction against different *Puccinia recondita* f. sp. *tritici* (*Prt*) races

Line No.	12-2	12-5	77-2	77-5	77A-1	104B	104-2	77-7	77-8	77-10
6	0;	0;	;	;	0;	0;	;	0;	;	;
7	0;	0;	;	;	0;	;	;	0;	0;	0;
36	0;	;	0;	;	0;	0;	;	0;	;	0;
142	0;	0;	;	0;	0;	0;	;	;	;	0;
143	0;	0;	0;	0;	0;	0;	;	0;	;	0;
144	0;	0;	0;	;	0;	0;	;	;	0;	0;
145	0;	0;	0;	;	0;	;	;	;	0;	0;
146	0;	0;	0;	;	0;	0;	;	;	0;	0;
147	0;	0;	;	;	0;	0;	;	0;	0;	0;

**Table 2** Accessions exhibiting immune reaction against different *Puccinia graminis* f. sp. *tritici* (*Pgt*) races

Line no.	11	21A-2	24A	40-2	117-3
43	0;	0;	0;	0;	0;
47	0;	0;	0;	;-	0;
50	0;	0;	0;	0;	0;
76	;	0;	;	;1	;
129	0;	0;	0;	2-	0;

germplasm lines (43, 47, 50, 76, 129) exhibited sufficiently high level of resistance against the pathotypes used. The wheat accessions were tested for resistance at seedling stage against ten virulent pathotypes of stripe rust. The pathotypes used in the study are 78S84, 46S119, 25A, T, K, L, P, A, N and 31. Based on the seedling resistance data 15 accessions (1, 2, 18, 28, 29, 48, 58, 61, 76, 99, 109, 125, 128, 139, 164) were found to be highly resistant (results presented in Table 3) against the tested pathotypes. The recently evolved pathotypes would also be used to screen these lines in coming crop season. Most of these accessions were characterized to have *Yr9* in gene postulation studies.

### Gene postulation based on SRT analysis

The SRT data of the test wheat accessions were compared with those of the differentials and the NILs with known *Lr* genes. The evaluated accessions mainly inferred *Lr13*, *Lr23* and *Lr26* genes, alone and in combinations. Gene postulation data obtained from gene matching technique is given in Fig. 1a. Among these genes, presence of *Lr13* was characterized in 38 genotypes. The presence of *Lr13* in combination with *Lr10*, *Lr1* and *Lr2* gene was recorded in 16,

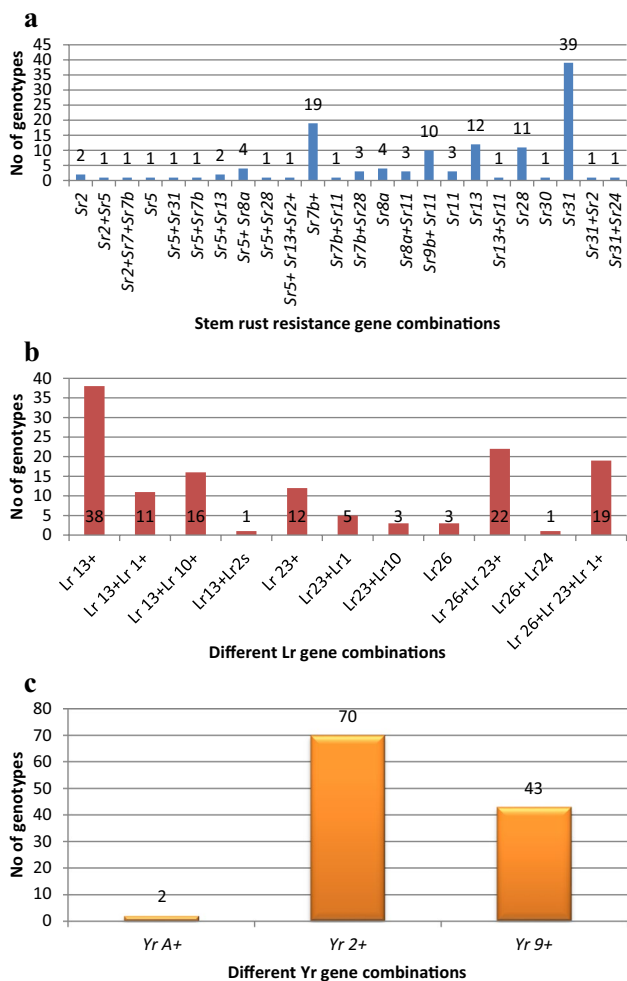
11 and 1 accessions respectively. *Lr23* was characterized in 12 genotypes whereas gene combination *Lr23 + Lr1* and *Lr23 + Lr10* was inferred in 5 and 3 genotypes respectively. The gene combination *Lr26 + Lr23 + Lr1* was characterized in 19 genotypes. Bhardwaj et al. (2010) in their study reported the presence of *Lr13* in combination with *Lr10*, *Lr1* in nine genotypes. *Lr13* is known to confer added resistance in combination with many resistance genes (Kolmer 1992).

The gene matching data revealed that these 166 accessions possess different stem rust resistance genes either present individually or in combination with other genes. The different gene combinations postulated in these accessions are given in Fig. 1b. *Sr31* gene was the most widespread stem rust resistance gene in these accessions. Virulence for stem rust resistance genes has been reported in different geographic areas (McIntosh et al. 1995). It is therefore of the primary importance to look for potential new sources of rust resistance to broaden the genetic diversity among new cultivars. Other resistance genes for stem rust *Sr7b*, *Sr13*, and *Sr28* gene were inferred in 19, 12 and 11 accessions respectively. *Sr5*, *Sr7b* and *Sr31* were found in combination with different *Sr* genes in these accessions. Although *Sr13* confers resistance to all the *Ug99* group races (typical infection types range from 2 to 2+) yet the resistance response is also influenced by prevailing temperature and genetic background (Roelfs and Mcvey 1979).

These accessions can be effectively used to develop stem rust resistant varieties either as alone or in combination with other resistance genes to develop cultivars containing durable resistance to stem rust. Multi-pathotype data at seedling stage for stripe rust resistance revealed that the tested wheat accessions were mainly characterized to have *Yr2* and *Yr9* gene (Fig. 1c). Out of the 166 test wheat accessions 70 inferred *Yr2* gene whereas 43 accessions were postulated to

**Table 3** Accessions showing resistance against different *Puccinia striiformis* f. sp. *tritici* (*Pst*) races

Line No.	46S119	78S84	P	K	L	31	N	T	20A	A
1	0;	;	0;	0;	0;	0;	0;	0;	0;	0;
2	0,	0,	0;	0;	0;	o,	0;	0;	0;	0;
18	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;
28	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;
29	0;	0;	;	;	0;	;	0;	0;	0;	0;
48	0;	;	0;	0;	0;	0;	0;	0;	0;	0;
58	0;	;	0;	;	0;	0;	0;	0;	0;	0;
61	;	0;	0;	0;	0;	0;	0;	0;	0;	0;
76	0;	;	0;	0;	0;	0;	0,	0;	0;	0;
99	0;	;	0;	;-	;-	0;	0;	0;	0;	0;
109	0;	;	0;	0;	0;	0;	0;	0;	0;	;-
125	;	0;	0;	0;	0;	0;	0;	0;	0;	;
128	;	0;	0;	0;	0;	0;	0;	0;	0;	0;
139	0;	0;	0;	0;	0;	0;	0;	0;	;-	0;
164	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;



**Fig. 1** a Gene postulation for stem rust based on seedling resistance. b Gene postulation for leaf rust based on seedling resistance. c Gene postulation for yellow rust based on seedling resistance

have *Yr9*. Only two accessions were characterized to have *YrA*.

**Rust resistance evaluation based on field screening (Adult Plant Resistance)**

Based on the screening of experimental material against the virulent and predominant pathotypes of stem and leaf rust pathogens at natural hotspot (Wellington), and under artificially created stripe rust epiphytotics at Karnal, following observations were recorded.

The data for Adult Plant Resistance (APR) on leaf rust was recorded at Wellington, which is a hotspot location, as infection occurs under natural conditions. Based on APR data 85 accessions were found completely resistant to leaf rust whereas 46 accessions were found susceptible. Among 166 accessions 25 were recorded as highly susceptible. Most of the accessions that resistance reaction at APR stage

may be carrying novel sources for resistance to leaf rust. Experiment for adult plant stem rust resistance was again conducted at Wellington under natural conditions. The disease scoring ranged from resistant (0) to highly susceptible (80S). Of the 166 germplasm lines, 96 were found to be resistant, 51 accessions were susceptible to stem rust and 16 accessions were highly susceptible. The APR resistance shown by 96 accessions may be a result of presence of different stem rust gene combinations. For stripe rust the artificial epiphytotic created at Karnal using a mixture of most virulent races led to identification of 16 lines which showed resistant reaction. Forty-seven germplasm lines were found to be susceptible with a disease severity of up to 40S. Out of 166 lines, 103 had a disease reaction of more than 40S and hence were categorized as highly susceptible. The lines conferring high level of resistance for stripe rust can be further characterized for resistance.

Based on the multi-environment APR data and the race specific seedling data, we were able to identify 28, accessions which were highly resistant to either one or two rusts. These lines are presented in Table 4 along with their pedigree details.

**Identification of lines resistant to spot blotch**

Spot blotch of wheat is an economically important disease in the eastern Indo-Gangetic plains of India. This disease can cause a huge loss to the wheat productivity if not managed. However, very few sources of resistance to this disease are known. The 166 exotic wheat accessions received from ICARDA were used to identify potential new sources of resistance. Sowing for screening of spot blotch resistant lines was done at Coochbehar (WB) and Wadura (J&K) and disease data were recorded for each accession. The recorded scores were categorized into three classes on the basis of infection scores; resistant (up to 25), moderately susceptible (26–57) and highly susceptible (> 57). Based on infection score, 26 accessions were considered to be resistant whereas, 90 accessions moderately susceptible with an infection score ranging from 25 to 57. Rest of the 40 accessions exhibited infection score > 57 and were classified as highly susceptible. The resistant accessions so identified can be further explored for characterization of the resistance origin.

**Conclusion**

The multi-environment evaluation of this set of exotic germplasm has led to the identification of lines with resistance to wheat rusts and also spot blotch. 28 accessions identified for resistance to the rusts and 26 lines for spot blotch can serve as a source for diversification of resistance in the Indian wheat germplasm. This work

**Table 4** Promising lines identified for different rusts and their pedigree details

Line no.	Pedigree
1	CHAM-4//SHUHA'S'/3/SD 8036
2	TURACO/CHIL/6/SERI 82/5/ALD'S'/4/BB/GLL//CNO67/7C/3/KVZ/TI
6	INQALAB 91/FLAG-2
7	INQALAB 91/FLAG-2
18	CLEMENT/ALD'S'//ZARZOUR/5/AU//KAL/BB/3/BON/4/KVZ//CNO/PJ62
28	IZAZ-2//TEVEE'S'/SHUHA'S'
29	FLORKWA-2//ASFOOR-5
36	NS732/HER//MILAN/SHA7
43	KAUZ/LUCO-M//PVN/STAR/3/FOW-1
47	DVERD-2//AE.SQUARROSA(214)//2*ESDA/3/NS732/HER
48	CBME4SA#4/FOW-2
50	MILAN/SHA7/3/NS732/HER//SUDAN #11
58	SAMAR-8//KAUZ'S'//CHAM-4/SHUHA'S'
61	SHUHA-8//VEE'S'/SAKER'S'
76	AGUILAL/FLAG-3
99	DAJAJ-1//VEE'S'/SAKER'S'
109	SERI 82/SHUHA'S'//DOVIN-2
125	MILAN/SHA7//POTAM*3KS811261-5/3/HAAMA-1
128	MILAN/YAMAMA
129	TURACO/CHIL/6/SERI 82/5/ALD'S'/4/BB/GLL//CNO67/7C/3/KVZ/TI
139	MARCHOUC 8/5/KAUZ/3/MYNA/VUL//BUC/FLK/4/MILAN
142	INQALAB 91/FLAG-2
143	ALD/CEP75630//CEP75234/PT7219/3/BUC/BJY/4/CBD/5/TNMU/PF85487
144	AGUILAL/FLAG-3
145	UTIQUE 96/FLAG-1
146	INQALAB 91/FLAG-2
147	MOR 1-72/3/PYN/BAU//MILAN
164	CHAM-4/SHUHA'S'/6/2*SAKER/5/RBS/ANZA/3/KVZ/HYS//YMH/TOB/4/BOW'S'

complements our previous study on identification of multiple disease resistant lines (Kumar et al. 2019) in a set of indigenous germplasm lines. The lines so identified in this study may be further used in the hybridization programs to diversify the resistance or these can be directly used in the plant breeding programs.

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