

Seedling and adult-plant stage resistance of a world collection of barley genotypes to stripe rust

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Abstract

Barley stripe rust caused by *Puccinia striiformis* f.sp. *hordei* (PSH) is one of the major diseases in barley production regions worldwide. A total of 336 barley genotypes with diverse genetic backgrounds targeted for low-input barley production were tested for seedling and adult-plant stage resistance against six PSH races (OS0, OS0-1, 1S0, 4S0, 5S0 and 7S0) originated from India. The seedling resistance was evaluated by inoculating the barley genotypes with six races separately under controlled conditions in Shimla, India. The same barley genotypes were evaluated for adult-plant stage resistance in the Agricultural Research Station (ARS) of Rajasthan Agriculture University, Durgapura, Rajasthan, India. Out of the 336 barley genotypes tested for seedling resistance, 119 (35.4%), 101 (30.1%), 87 (25.9%), 100 (29.8%), 91 (27.1%) and 70 (20.8%) genotypes were resistant to races OS0, OS0-1, 1S0, 4S0, 5S0 and 7S0, respectively. In the field, 102 (30.3%) genotypes showed the resistance response of which 18 (5.3%) genotypes were highly resistant to PSH. Barley genotypes AM-14, AM-177, AM-37, AM-120, AM-300, AM-36, AM-103, AM-189, AM-291, AM-275 and AM-274 showed resistance response to all six races at seedling and adult-plant stages. Seedling resistance reported in the current study is effective against the newly emerged race 7S0 and previously reported five races in India. Therefore, resistant barley genotypes identified in the current study provided effective protection against all six races at seedling and adult-plant stages. The stripe rust resistance identified in the current studies may be potential donors of stripe rust resistance to barley breeding programmes in India and elsewhere.

KEYWORDS

barley, race, resistance, stripe rust, yellow rust

1 | INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the most important cereal crops grown on more than 49.7 million ha (FAOStat 2015) and is mainly used as feed, food and malt in many countries (Newman & Newman, 2006). This cereal is adapted to dry areas characterized

by erratic rain and poor soil fertility which are often described as low-input barley (LIB) production systems. Biotic stresses, mainly stripe rust (caused by *Puccinia striiformis* Westend. f.sp. *hordei* Erikss.) (PSH), cause significant yield losses in barley. Stripe rust often causes serious epidemics in South Asia (India, Nepal and Pakistan) (Bahl & Bakshi, 1963; Bakshi, Bahl, & Kohli, 1964; Luthra

& Chopra, 1990; Murty, 1942; Pradhanang & Sthapit, 1995; Upreti, 2005; Vaish, Ahmed, & Prakash, 2011), West Asia (Safavi, 2012), East Africa (Stubbs, 1985; Woldeab, Fininsa, Singh, & Yuen, 2007), South America (Capettini, 2005; Stubbs, 1985) and North America (Chen, 2007, 2008; Chen & Line, 2002; Chen, Line, & Leung, 1995; Dubin & Stubbs, 1986; Line, 2000 and Roelfs & Huerta-Espino, 1994). Frequent and serious stripe rust epidemics caused significant yield loss ranging from 5% to 25% in wheat and barley while yield loss as high as 70% was reported in barley in South America (Wellings, 2011).

Deployment of durable resistance is the most profitable, cost effective and environmentally sound strategy to manage the rust disease (Park, 2008). In cereal rusts, two major types of resistances have been described, including seedling/all-stage resistance and adult-plant resistance (APR). It has been demonstrated that all-stage resistance is effective throughout all stages of plant growth, which is often characterized by the hypersensitive type of responses while the APR is effective only at adult-plant stage, and is often regarded as slow rusting (Park, 2008). Recently, a new stripe rust race, 7S0 was reported in 2014 which overcomes seedling stage resistance of barley cultivars effective against races prevalent in India. Several studies have been reported on seedling and APR resistance in barley leaf rust caused by *Puccinia hordei* whereas information on APR to stripe rust is still scant. For example, several *P. hordei* all-stage resistance genes conferring high level of resistance, including *Rph1-Rph19* (Golegaonkar, Singh, & Park, 2009), *Rph21* (Sandhu et al., 2012) and *Rph22* (Johnson, Niks, Meiyalaghan, Blanchet, & Pickering, 2013), have been characterized. Recently, Dracatos et al. (2016) and Esvelt Klos et al. (2016) reported QTL mapping of PSH resistance at seedling stage using European and North American PSH races, respectively. Often, all-stage resistance genes are dominant in nature with large effects. Frequent mutations in rust virulence genes often lead to the breakdown of corresponding major resistance genes in the host within a short period of deployment (Park, 2008). In contrast, APR is mostly quantitative in nature, is often referred to as incomplete or slow rusting and is often additive in nature (Carlborg & Haley, 2004; Golegaonkar et al., 2009; Singh, Dracatos, Derevnina, Zhou, & Park, 2015). Therefore, APR genes are more often effective for a longer period.

The APR genes are less studied in barley due to their partial mode of action. Verma et al. (2016) reported seedling (against five races) and adult-plant stage resistance to stripe rust in genotypes originating from high-input barley breeding programme of the International Center for Agricultural Research in the Dry Areas (ICARDA). They identified 12 stripe rust-resistant genotypes against five PSH races in India. However, information on APR genes against PSH races from barley is still inadequate. Among PSH reported in India, race 24 has been widely reported in major barley-growing regions across the globe (Chen, 2007) while other PSH races used by Verma et al. (2016) are endemic to India. Therefore, the objective of this study was to identify sources of seedling and APR in barley genotypes adapted to LIB breeding programmes to Indian PSH races, including the newly emerged race 7S0.

2 | MATERIALS AND METHODS

2.1 | Barley genotypes and stripe rust races

A world collection of association mapping (AM-2014) panel of 336 barley genotypes with diverse sources (Table S1) was assembled for the LIB breeding programme of ICARDA. The genetic diversity and detail descriptions of AM-2014 were reported by Amezrou et al. (2017). In brief, out of 336 barley genotypes, 230 genotypes were collected from the LIB breeding programme (genotypes adapted for abiotic and biotic stress tolerances) and 82 from the high-input barley breeding programme (genotypes adapted to favourable conditions) of ICARDA and the remaining 26 were frequently used in both programmes (Table S1). Based on grain types, 276 genotypes were hulled and 60 were hull-less barley. In terms of row type, 137 genotypes were two-rowed and 199 were six-rowed. The majority (73.8%) of the barley genotypes was collected from barley breeding programmes of ICARDA (advanced breeding lines), but also represented genotypes from different sources, including the Genetic Resource Unit (Gene Bank) of ICARDA (9.5%) and barley varieties released by breeding programmes (16.6%) from India, Australia, USA, Canada and Morocco. Apart from a few genotypes that originated from Indian breeding programmes, most genotypes in the AM-2014 had never been tested for reactions to Indian PSH races. The AM-2014 was evaluated for PSH races because several genotypes included in this panel furnish crossing block of the LIB programme of ICARDA targeted for feed and food barley improvement across the globe.

The AM-2014 was evaluated for seedling resistance under controlled glasshouse conditions at Indian Institute of Agricultural Research (ICAR)-Indian Institute of Wheat and Barley Research (IIW&BR), Regional Station, Shimla, India. Five common PSH races [(57 (0S0), 24 (0S0-1), M (1S0), G (4S0) and Q (5S0)] and a recently reported race, 7S0, were used to evaluate seedling resistance.

2.2 | Seedling stage evaluation of resistance to stripe rust in the glasshouse

The seedling resistance of 336 barley genotypes was evaluated to each of the six PSH races, 57 (0S0), 24 (0S0-1), M (1S0), G (4S0) Q (5S0) and 7S0 at ICAR-IIW&BR, Shimla, India, following the methods described by researchers (Nayar, Prashar, & Bhardwaj, 1997; Prashar, Bhardwaj, Jain, & Datta, 2007; Verma et al., 2016; Zadoks, 1961). In brief, aluminium trays 29 cm long × 12 cm wide × 7 cm deep were filled with a mixture of fine loam and farmyard manure (3:1). Twenty holes (10 holes in each row, 4 cm deep and 5 cm apart) were made with the help of wooden marker in the soil bed. Five seeds of a test genotype were sown in each hole, and 18 genotypes were seeded in one tray. In each tray, the susceptible check "Bilara-2" was included at locations of 7th and 14th holes. Bilara-2 does not contain any known PSH resistance against any races known so far in India. The seedlings were raised in glasshouse chambers at 22 ± 2°C, 50%–70% relative humidity and 12-hr daylight cycle. One-week-old seedlings with fully expanded primary leaves were inoculated with 100 mg spores of individual races suspended in 10 ml light grade mineral oil (Soltrol 170; Chevron Phillips

Chemicals Asia Pvt. Ltd., Singapore). The inoculated seedlings were kept for 48 hr in dew chambers at $16 \pm 2^\circ\text{C}$ with $>90\%$ relative humidity and 12 hr of the day/night cycle. The plants were then transferred to glasshouse benches and incubated at $16 \pm 2^\circ\text{C}$ with $>70\%$ relative humidity, illuminated at approximately 15,000 lux for 12 hr. Powdery mildew was controlled by spraying sulphur powder.

Reactions of genotypes as infection types (IT) to rust infection were recorded 16–18 days after inoculation following the modified method (Nayar et al., 1997; Stakman, Stewart, & Loegering, 1962): where 0; (naught fleck) = no visible infection,;- (fleck minus) = slightly necrosis / microflecking visible,; (fleck) = no uredia but small hypersensitive flecks present, 1 (one) = uredia minute, surrounded by distinct necrotic areas, 2 (two) = small to medium uredia surrounded by chlorotic or necrotic boarder, 3 (three) = uredia small to medium in size and chlorotic areas may be present, 3+ (three+) = uredia large with or without chlorosis, sporulating profusely and forming rings. Infection type 33+ is classified when both 3 and 3+ pustules occur together. A pictorial view of these ITs is presented in Figure S1. The experiment was repeated two times. In repeated experiments, the majority of ITs were consistent except very few cases where susceptible ITs were kept over resistance ITs. The ITs 0 to 2 ratings were considered resistant and 3 to 3+ as susceptible while 2+, 22+ and 3- were considered intermediate ITs.

2.3 | Adult-plant stage evaluation of resistance to stripe rust in the field

All genotypes screened for seedling resistance were also screened for adult-plant stage resistance to stripe rust at the ARS of Rajasthan Agricultural University (RAU) Durgapura ($75^\circ 47' \text{ E}$, $26^\circ 51' \text{ N}$), Rajasthan (RJ), India, in the 2014–2015 cropping season. The experiment was laid out in an augmented design where the susceptible check, Bilara-2, was repeated in each block of 20 test genotypes. Seeds were sown in one-metre rows with 25-cm row to row distance for each genotype on 15 November 2015. Bilara-2 was sown as spreader perpendicular to the plots throughout the experimental blocks and around the perimeter of the test blocks 15 days before the sowing of experimental genotypes. Stripe rust epidemic was created by inoculating a mixture of the six PSH races, including 57 (0S0), 24 (0S0-1), M (1S0), G (4S0), Q (5S0) and 7S0 received from ICAR-IIW&BR Shimla, India. These races were mixed in equal amount before inoculation. The spreader plots were first syringe inoculated at Zadoks GS 10-19 (21 days of seedling stage) (Zadoks, Chang, & Konzak, 1974) with the mixed inocula of races followed by repeated sprays of inocula collected from spreader rows onto the test genotypes. Irrigations were carried out as required to maintain sufficient humidity for better rust infection. Disease severity and reactions were recorded three times at Zadoks 60-69 growth stages.

A modified Cobb scale (Peterson, Campbell, & Hannah, 1948) was used in the field to assess stripe rust severity and host reactions. Host responses were recorded as R = no uredia present; TR = trace or minute uredia on leaves without sporulation; TMR = trace or minute uredia on leaves with some sporulation; MR = small uredia with slight sporulation; MR-MS = small-to-medium-sized uredia with moderate

sporulation; MS-S = medium-sized uredia with moderate to heavy sporulation; and S = large uredia with abundant sporulation, uredia often coalesced to form lesions as described by Roelfs, Singh, and Saari (1992). The coefficient of infection (CI) was calculated by using disease severity and host response according to Stubbs, Prescott, Saari, and Dubin (1986). Area under the disease progress curve (AUDPC) was calculated using CI of disease severity data recorded three times at 10-day intervals.

$$\text{AUDPC} = \sum_{i=1}^n [(C_{i+1} + C_i) / 2] [(t_{i+1} - t_i)]$$

where, C_i = Coefficient of Infection as defined above on i^{th} days, t_i = time in days at i^{th} observation, and n = total number of observations.

2.4 | Statistical analysis

The adult-plant stage rust severity was subjected to ANOVA using augmented block design. The ANOVA was performed using PROC GLM of the SAS (SAS Institute 1988) statistical software package. The AUDPC of barley genotypes was differentiated by Fisher's least significant difference (LSD) ($p = .05$) based on the standard error of the mean difference of 17 repeated checks, Bilara-2, that was used in the experiment. The cut-off of rust resistance and susceptible genotype was 245.7 AUDPC which was determined by significant t test of Bilara-2 and test genotypes at 0.05 probability [AUDPC = 162 ($p < .05$)] plus $\text{LSD}_{0.05}$ which was AUDPC = 83.7. Therefore, genotypes with rust severity lower than the cut-off AUDPC 245.7 were considered resistance and vice versa.

3 | RESULTS

The ITs of stripe rust on barley genotypes evaluated at seedling stage are presented in Table S1. Of the total genotypes evaluated, 35.4%, 30.1%, 25.9%, 29.8%, 27.1% and 20.8% genotypes showed resistance reactions to the races 57 (0S0), 24 (0S0-1), M (1S0), G (4S0), Q (5S0) and 7S0, respectively (Table 1). Among these genotypes, 91 (20.8%) genotypes were resistant (R) and had ITs of either 0, '1, 2 or 2- and 12 (3.6%) genotypes were moderately resistant (MR) and had ITs of 2+, 22+ or 3- to race 7S0. In contrast, 225 (67%) genotypes were susceptible (S) ITs (3, 33+ or 3+) to 7S0. The ITs of barley genotypes to other previously reported races are also presented in Table 1.

The AUDPC of the 336 barley genotypes screened in the field is presented in Table S1 and Figure 1. The ANOVA of AUDPC of rust severity is presented in Table 2. Highly significant ($p < .001$) effects of genotypes were found on rust severity at adult-plant stage. Based on ITs at seedling stage and AUDPC cut-off (<245.5) for resistance reactions, nine genotypes, namely AM-14, AM-177, AM-37, AM-120, AM-300, AM-36, AM-103, AM-189 and AM-291, showed resistance in both seedling and adult-plant stages (Table 3). Bilara-2 showed highly susceptible reaction with 100S severity at 65-69 Zadoks GS and AUDPC LS mean was 3,282.2. In contrast, five genotypes (Group 2) showed resistance IRs to all six races in seedling but showed

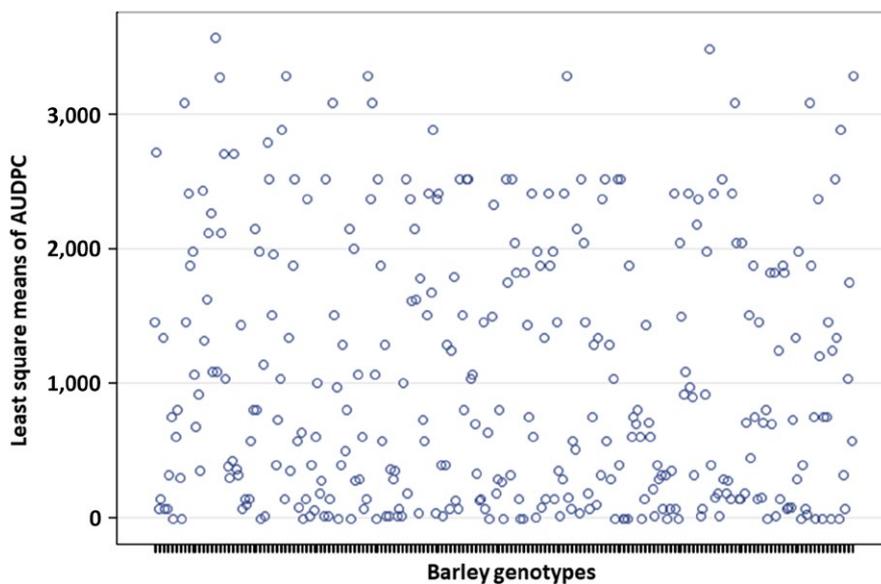
TABLE 1 Seeding reactions of barley genotypes ($n = 336$) to six *Puccinia striiformis* f.sp. *hordei* races under controlled conditions in glasshouse in 2015 in Shimla, India

Infection Type	Number of genotypes					
	57 (0S0) ^e	24 (0S0-1) ^e	M (1S0) ^e	G (4S0) ^e	Q (5S0) ^e	7S0 ^e
'0' '1'	60 (17.9)	65 (19.3)	42 (12.5) ^f	22 (6.5)	49 (14.6)	58 (17.3)
'1'	0 (0)	0 (0)	0 (0)	22 (6.5)	0 (0)	0 (0)
'2' '2-'	59 (17.6)	36 (10.7)	45 (13.4)	56 (16.7)	42 (12.5)	12 (3.6)
Resistant ^a	119 (35.4)	101 (30.1)	87 (25.9)	100 (29.8)	91 (27.1)	70 (20.8)
'3'	33 (9.8)	16 (4.8)	17 (5.1)	104 (31)	51 (15.2)	32 (9.5)
'33+' '3+'	125 (37.2)	172 (51.2)	194 (57.7)	72 (21.4)	137 (40.8)	193 (57.4)
Susceptible ^b	158 (47)	188 (56)	211 (62.8)	176 (52.4)	188 (56)	225 (67)
Intermediate ^c	33 (9.8)	14 (4.2)	15 (4.5)	19 (5.7)	30 (8.9)	12 (3.6)
NT ^d	26 (7.7)	33 (9.8)	23 (6.8)	41 (12.2)	27 (8)	29 (8.6)

^aNumber of genotypes showing resistant infection type (IT) '0' '1' '2' '2-'.
^bNumber of genotypes showing susceptible infection type '3' '33+' '3+'.
^cIntermediate infection types were considered as '2+' '22+' '3-'.
^dNot tested due to poor germination.

^eStripe rust races used in the study.

^fNumber of genotypes, values in the parentheses are percentage.

**FIGURE 1** Least square (LS) mean of area under the disease progress curve (AUDPC) of 336 barley genotypes to stripe rust (*Puccinia striiformis* f.sp. *hordei*) in Durgapura, Rajasthan, India. Disease severity and infection types were recorded three times (at the interval of 10 days) at Zadoks GS 60-69 on barley leaves, and area under the disease progress curve (AUDPC) was calculated using coefficient of infection (CI). The AUDPC LS mean of Bilara-2 (repeated susceptible check) was estimated as 3,282.2**TABLE 2** Analysis of variance of area under the disease progress curve (AUDPC) of stripe rust severity in 336 barley genotypes

Source of variation	df	SS	MS	p-Value
Block	8	4271995.5	533999	<.0001
Genotype	335	380209737	1134954	<.0001
Error	7	35535.9	5076.6	

Coefficient of variation (CV) = 6.3%.

susceptible reaction to the mixture of six PSH races (AUDPC ranged from 1,350 to 3,100) at adult-plant stages in the field.

The evaluation of adult-plant stage resistance revealed that 18 genotypes were immune (I), 26 genotypes highly resistant (HR), 58

R, 91 MR, 77 moderately susceptible (MS), 54 S and 10 highly susceptible (S) (Figure 1). In total, 102 (30.5%) genotypes were resistant, 141 (42.2%) genotypes were susceptible while the rest 27% genotypes were either MR. The APR to the mixture of the six PSH races is presented in Table 4. Of the 336 genotypes, 88 genotypes that showed susceptible ITs at seedling stage to at least one or more races, but were found resistance at adult-plant stage in the field. The AUDPC severity in these 88 genotypes ranged from 0 to 218. It was interesting to note that 16 genotypes which showed susceptible ITs (3, 33+ or 3+) to one or multiple races at seedling screening showed highly resistance reaction (AUDPC = 0) at adult-plant stages (Table 4). Among the 89 genotypes which showed APR, 68 genotypes showed disease severity of <20R, <20MR or <20MS while AUDPC ranged from 3.4 to 162. However, seven genotypes

TABLE 3 Resistance reactions of barley genotypes to six *Puccinia striiformis* f.sp. *hordei* races at seedling and adult-plant stages screenings in 2015 in India

Genotypes	Stripe rust infection type in seedling ^a						Adult-plant stage ^b	
	M (150)	24 (050-1)	57 (050)	G (450)	Q (550)	750	Severity	AUDPC
Group 1 ^c								
AM-14	2-	0;	2	-	0;	-	0	0
AM-177	2	;	2	2C	2+	;	0	0
AM-37	;	-	0;	2-	2+	0;	5MR	24.4
AM-120	0;	0;	2-	0;	2	0;	5MR	24.4
AM-300	-	0;	;	-	2	0;	5MR	24.4
AM-36	0;	0;	0;	;-	0;	0;	20MR	109
AM-103	2-	;	0;	0;	0;	0;	20MR	146
AM-189	;	2	0;	2N	2	0;	10MS	150
AM-291	2-	2-	2-	1CN	-	-	10MS	150
Group 2 ^d								
AM-188	2	0;	2	1	-	0;	60S	1,350
AM-283	0;	-	2-	0;	2+	0;	100S	2,060
AM-261	2-	2	2-	2-	2	0;	80S	2,190
AM-173	2	2	2-	2-	2-	0;	100S	2,530
AM-87	2-	2	2	1C	2	2+	100S	3,100
Bilara-2 ^e	3+	3+	3+	3+	3+	3+	100S	3,282

Bold faced genotypes are also resistant to leaf and stem rust races at seedling stage in India.

^aSeedling resistance testing (SRT) using six stripe races in Rust Research Station, ICAR-IIW&BR, Shimla, India. C = pronounced chlorosis, N = pronounced necrosis, CN = both necrotic and chlorotic area present with rust pustules, - = not tested due to poor germination.

^bStripe rust resistance evaluated at adult-plant stage in Durgapura Research Station, Rajasthan, India. The area under the disease progress curve (AUDPC) was calculated for stripe rust severity. The CV = 6.3% and LSD_{0.05} = 83.7 for AUDPC were estimated using Proc. GLM in SAS.

^cGroup 1—barley genotypes with seedling and adult-plant stage resistance to stripe rust. The pedigrees of the barley genotypes are listed below.

AM-14 = GK58/3/Kc/MullersHeydla//Sl/4/Wieselbuger//Ahor1303-61//Ste/Antares.

AM-36 = PENCO/CHEVRON-BAR/3/LEGACY//PENCO/CHEVRON-BAR.

AM-37 = PENCO/CHEVRON-BAR/3/LEGACY//PENCO/CHEVRON-BAR.

AM-103 = Arar/H.spont.19-15//Hml/3/H.spont.41-1/Tadmor/4/Barque.

AM-120 = ArabiAbiad/Arar//H.spont.41-5/Tadmor/3/ArabiAbiad/Arar//H.spont.41-5/Tadmor.

AM-177 = Rihane-03/3/As46/Aths*2//Aths/Lignee686/4/Alanda-01.

AM-189 = Avt/Attiki//M-Att-73-337-1/3/Aths/Lignee686/4/CYDBA89#49/3/Ssn/Bda//Arar.

AM-291 = IG: 153849 (landrace from Nepal).

^dGroup 2—seedling resistance but susceptible to adult stage.

^eBilara-2 was a stripe rust susceptible check.

showed either 10S or 20 MS reaction and AUDPC of these genotypes was either 187.5 or 218. Bilara-2, the susceptible check repeated multiple times in the experiment, always recorded 3+ IT to all six races at seedling and rust severity of 100S or AUDPC = 3,282.2 at adult-plant stages.

4 | DISCUSSION

In this study, we have reported stripe rust resistance of spring barley genotypes originated from ICARDA to Indian PSH races. Nearly 21% (70 out of 336) genotypes showed a high level of resistance to recently reported virulent race 750. Stripe rust resistances identified in this study are valuable genetic resources for the barley breeding programme in the subcontinent and elsewhere. Specifically, stripe

rust is one of the major production constraints in barley production in Asian countries including India, Nepal and Pakistan (Bahl & Bakshi, 1963; Chen et al., 1995; Luthra & Chopra, 1990; Verma et al., 2016). Vaish et al. (2011) reported that PSH was the major foliar disease reported in trans-Himalayan Ladakh region of India with >45% PSH prevalence in the field. Similarly, the most popular barley cultivar “Solu Uwa” is reported highly susceptible to stripe rust causing 30% yield loss in Nepal (Upreti, 2005). Several PSH-resistant cultivars were released periodically in India in last two decades. However, the effectiveness of PSH resistance is limited to India due to the frequent emergence of new races and the breakdown of seedling and all-stage resistance (Verma et al., 2016). Chen (2007, 2008) reported that 22 new PSH isolates were detected since 2002 in the USA while 74 new races were reported since 1995–2005. The emergence of new PSH races was due to changes in the virulence spectrum of

TABLE 4 Barley genotypes showing adult-plant resistance (APR) to *Puccinia striiformis* f.sp. *hordei* in 2015 in Durgapura, Rajasthan, India

Genotype ^a	Infection type at seedling stage ^b					Adult-plant severity ^c	Infection type at seedling stage ^b					Adult-plant severity ^c	
	ISO	050-1	050	450	550		750	ISO	050-1	050	450		550
AM-52	2	0;	0;	;-	3+	0;	0 (0) ^d	3+	- ^e	0;	3	3+	10MR (75)
AM-73	2	33+	2	3-	3	0 (0)	0 (0)	3+	33+	0;	2+	2-	10MR (75)
AM-90	3+	3+	33+	3	33+	0 (0)	0 (0)	0;	33+	2	3N	3+	10MR (75)
AM-108	3+	;	2+	3C	2+	0 (0)	0 (0)	3+	3+	33+	3	3+	10MR (75)
AM-162	3+	0;	3+	3	3-	0 (0)	0 (0)	2+	;	2+	1C	0;	10MR (75)
AM-169	3+	3+	3+	3+	3+	0 (0)	0 (0)	3+	2	2	2	33+	10MR (75)
AM-178	- ^e	3	0;	-	2	0 (0)	0 (0)	3	0;	-	3	0;	10MR (75)
AM-222	3	33+	33+	3	3+	0 (0)	0 (0)	0;	3+	3+	3	3+	10MR (75)
AM-226	3+	3	3+	-	3+	0 (0)	0 (0)	;	-	3	3	-	10MR (75)
AM-228	3+	33+	3+	3+	3	0 (0)	0 (0)	3	2+	2	1C	2+	10MR (75)
AM-235	3+	3+	3	3	;	0 (0)	0 (0)	2+	33+	2+	3	33+	10MR (75)
AM-247	2	33+	3	3	33+	0 (0)	0 (0)	3+	-	-	-	-	10MR (75)
AM-252	3	;-	0;	2	;	0 (0)	0 (0)	3+	-	0;	22+	0;	10MS (82.8)
AM-312	0;	0;	-	-	3+	0 (0)	0 (0)	3+	2	2+	2-	3+	10MS (82.8)
AM-319	2-	33+	33+	3	2+	0 (0)	0 (0)	3+	3+	2-	3C	3+	10MS (82.8)
AM-322	2	;	2	3	3	0 (0)	0 (0)	33+	0;	2	2-	3	10MS (82.8)
AM-10	3+	3+	3+	3	3+	TMR (3.4)	TMR (3.4)	33+	33+	2	22+	3+	10MS (82.8)
AM-96	33+	2	33+	3	3	TMR (3.4)	TMR (3.4)	3+	3	2+	3C	3+	20MR (109)
AM-227	3+	3+	3+	3+	3+	TMR (3.4)	TMR (3.4)	3+	0;	0;	3+	0;	10MR (111.5)
AM-296	3+	3+	33+	3+	3	TMR (3.4)	TMR (3.4)	3+	2-	3+	3+	3+	10MS (142)
AM-326	33+	33+	33+	2	3+	TMR (3.4)	TMR (3.4)	0;	-	0;	2-	0;	15MR (145)
AM-330	3+	3	2	3	0;	3+	3+	3+	2	3	2	0;	20MR (146)
AM-184	3+	3	2-	3+	2+	5MR (17)	5MR (17)	3	3+	3	3	3	20MR (146)
AM-76	3+	3+	;	3N	0;	5MR (20.7)	5MR (20.7)	3+	3	0;	2-	3+	20MR (146)
AM-196	0;	3+	33+	3	33+	5MR (20.7)	5MR (20.7)	3+	;	0;	3	0;	10MS (146)
AM-54	33+	0;	-	1C	2+	5MR (24.4)	5MR (24.4)	2-	2	3	;	33+	20MR (146)
AM-83	2	;-	;	1C	;	5MR (24.4)	5MR (24.4)	3+	33+	3+	3+	33+	10MS (150)
AM-85	3+	3+	33+	3	3+	5MR (24.4)	5MR (24.4)	3+	3+	3+	3	3+	10MS (150)
AM-113	3+	0;	3	3	33+	5MR (24.4)	5MR (24.4)	33+	3	2	3	3	10MS (150)

(Continues)

TABLE 4 (Continued)

Genotype ^a	Infection type at seedling stage ^b						Adult-plant severity ^c	Infection type at seedling stage ^b						Adult-plant severity ^c	
	ISO	OSO-1	OSO	450	550	750		ISO	OSO-1	OSO	450	550	750		
AM-114	3+	33+	3+	33+	33+	3+	5MR (24.4)	AM-193	33+	3+	3+	3C	3	3+	10MS (150)
AM-118	3+	3+	3+	3	3	3+	5MR (24.4)	AM-277	;	33+	3+	3+	3	3+	10MS (150)
AM-241	3	33+	2+	3+	2	0;	5MR (24.4)	AM-281	3+	3+	3+	3	3	3+	10MS (150)
AM-263	-	2+	2	3	0;	-	5MR (24.4)	AM-302	3+	3+	33+	-	-	33+	10MS (150)
AM-272	0;	0;	3+	;	0;	0;	5MR (24.4)	AM-236	3+	33+	0;	3	2+	3+	10MS (152)
AM-315	3+	3+	3+	3	3+	3+	10R (37.5)	AM-200	3+	3+	3+	3+	3+	3+	20MR (162)
AM-128	2	0;	2-	1N	2	3+	10MR (41.4)	AM-270	3+	3+	3+	3	3+	3+	20MR (162)
AM-205	0;	3+	0;	3+	3+	3	10MR (41.4)	AM-293	33+	3+	3+	33+	3+	3+	20MR (162)
AM-78	0;	3+	33+	3C	3+	3+	5S (61)	AM-81	33+	33+	0;	2N	3	3+	10S (187.5)
AM-333	3+	3+	3+	3+	3+	3+	10MR (71)	AM-123	3+	3+	2	-	0;	-	10S (187.5)
AM-7	2	-	0;	2	3+	3+	10MR (73)	AM-165	3+	3	3	33+	3	3+	10S (187.5)
AM-102	3+	3+	33+	33+	3+	3+	10MR (73)	AM-209	33+	22+	;	3	2+	3+	10S (187.5)
AM-160	33+	33+	33+	3	2+	3+	10MR (73)	AM-271	2-	3+	33+	3N	3	3+	10S (187.5)
AM-3	3	2+	0;	2	33+	3+	10MR (75)	AM-284	3+	0;	33+	3+	3+	3+	10S (187.5)
AM-6	3-	3+	33+	3	3	3+	10MR (75)	AM-240	3+	0;	2+	3+	3	0;	20MS (218)
AM-23	2-	2	2-	2N	2+	3+	10MR (75) ^d								
Bilara-2 ^f	3+	3+	3+	3+	3+	3+	100S (3,282)	Bilara-2 ^f	3+	3+	3+	3+	3+	3+	100S (3,282)

^aBarley genotypes.

^bInfection type of barley genotypes at seedling stage after challenged with six stripe rust races under controlled conditions in the glasshouse in Shimla, India.

^cStripe rust severity recorded at adult-plant stage to screen resistance. Values in the parentheses are area under the disease progress curve of stripe rust severity. The CV = 6.3% and LSD_{0.05} = 83.7 were estimated using Proc. GLM in SAS.

^dStripe rust severity recorded at third reading, values in the parentheses is the area under the disease progress curve (AUDPC) calculated using coefficient of infection estimated from stripe rust severity recorded at three dates after postflowering growth stages (Zadoks GS 60-69) in Durgapura, Rajasthan, India.

^eData not recorded due to poor seed germination.

^fBilara-2 was a stripe rust susceptible check and was repeated 18 times in both seedling screening in the glasshouse in Flowerdale, Shimla, and adult-plant stage screening in the field in Durgapura, Rajasthan, India.

stripe rust (Chen, 2007). Kumar, Holtz, Xi, and Turkington (2012) reported highly diverse PSH pathotypes from Canada compared to isolates reported in the past. The emergence of new PSH race 750 in India was consistent with previous reports (Chen, 2007, 2008; Kumar et al., 2012). Genotypes AM-177, AM-37, AM-120, AM-300, AM-36, AM-130, AM-189 and AM-274 provided resistance to newly evolved virulent race 750 at seedling and adult-plant stage besides previously reported PSH races. Therefore, the identification of resistance sources in low-input genotypes, in the current study, will provide protection against major PSH races currently prevalent in India.

Genotypes AM-14, AM-177, AM-37, AM-120, AM-300, AM-36, AM-130, AM-189, AM-291 and AM-274 showed resistance at both seedling and adult-plant stages. Park (2008) suggested that when genotypes show rust resistance at both seedling and adult-plant stages, it can be referred to as all-stage resistance. Possibly, these genotypes might have all-stage resistance to PSH races prevalent in India. The seedling resistance is not growth stage-dependent (Park, 2008; Singh, 1992; Singh et al., 2015). However, seedling resistance does not always provide protection against rust at adult-plant stages. Our data also suggested that genotypes AM-87, AM-173, AM-188, AM-261 and AM-283 possessed seedling resistance, but failed to protect from PSH, with AUDPC >218, at adult-plant stage. Therefore, a genotype with stripe rust resistance at seedling stage alone is not sustainable and effective for a long-term deployment (Park, 2008; Singh, 1992; Singh et al., 2015). Often, seedling resistance is governed by major gene(s) and frequent mutations in corresponding avirulence genes in the rust pathogen may lead to catastrophic failure of the crop (Park, 2008). Therefore, identification of any new sources of resistance to new PSH races is extremely important for barley breeding programmes. The central barley breeding programme of ICAR-IIW&BR at Karnal as well as several regional barley breeding programmes in India will immediately benefit from the currently identified stripe rust resistances in this study.

Eight genotypes, resistance to PSH race 750 that is identified in the current study, have diverse pedigrees. AM-36 and AM-37 are sister lines and share a common pedigree (PENCO / CHEVRON-BAR /3/ LEGACY // PENCO / CHEVRON-BAR); however, the donor plant is unknown. Genotypes AM-103 and AM-120 also share common parentages apart from one wild barley accession. The AM-103 contains two wild accessions in its pedigree, *Hordeum spontaneum* 19-15 and *H. spontaneum* 41-5 (IG_138213) while AM-120 has *H. spontaneum* 41-5 only. Among these, two wild accessions, IG_138213 is one of the important sources of drought tolerance in the LIB programmes of ICARDA. We do not know which wild accessions contributed to PSH resistance in these two resistant genotypes. Therefore, further research is warranted to study PSH 750 resistance in these wild accessions. Similarly, AM-177 and AM-189 also share common parentage, but their ITs were different than other resistant genotypes. Possibly, these genotypes may carry different resistant gene(s), but further research on allelic relationship of these resistance sources is needed to verify the nature of these resistance sources.

The APR to Indian PSH races reported in this study is unique. Of 88 genotypes, which showed a high level of APR, 16 genotypes exhibited immune (AUDPC = 0) responses at adult-plant stage screening. Verma et al. (2016) reported that weather conditions in Durgapura, RJ, favours the stripe rust development in barley compared to Karnal and other locations in India. The weather conditions, temperature and humidity in 2014–2015 growing season (data not presented) were favourable for stripe rust infection, rust development and secondary spreads of stripe rust urediniospores from spreader rows to test genotypes. As Bilara-2 consistently scored 100S and an ab average AUDPC of 3,282 on all 17 repeated plots, the 16 lines that showed immune responses are likely due to strong resistance. Park (2008), Carlborg and Haley (2004), Golegaonkar et al. (2009); Singh et al. (2015) and Singh (1992) reported that APR is conditioned by additive genes; therefore, phenotypic responses of APR genes are generally quantitative in nature. Similarly, the adult-plant stage PSH-resistant genotypes reported by Safavi (2012) exhibited slow rusting responses which suggested that PSH resistance was quantitative in nature. In this study, the immune response of these 16 genotypes, at adult-plant stage screening, was unique in nature and requires further genetic studies to elucidate nature of PSH resistance. However, this result was consistent with immune type of stripe rust resistance at the adult-plant stage reported by Verma et al. (2016) in India. In wheat, several reports are available where immune or higher level of APR has been reported (Milus, Moon, Lee, & Mason, 2015; Sørensen, Hovmøller, Leconte, Dedryver, & Vallavieille-Pope, 2014). Milus et al. (2015) described these APRs as race-specific APR in winter wheat. The 89 barley genotypes with higher level of APR reported in this study showed susceptible IT to at least one PSH race at seedling stage, but recorded AUDPC ≤218 (Table 4). Therefore, these genotypes were able to slow down the stripe rust infections at adult-plant stage, which were in agreement with previously reported APR to stripe, leaf and stem rusts in barley (Carlborg & Haley, 2004; Golegaonkar et al., 2009; Park, 2008; Singh, 1992; Singh et al., 2015) and APR to stripe rust in wheat (Hickey et al., 2011; Milus et al., 2015; Sørensen et al., 2014). The APR genotypes identified in this study are valuable resources of PSH resistance and can provide effective and durable resistance against PSH particularly if they are combined with seedling resistance. The marker-trait association studies using 9K iSelect Illumina Infinium SNPs chip and stripe rust resistance to the six races at seedling and adult-plant stages are in progress.

ACKNOWLEDGEMENTS

The authors sincerely acknowledge internal reviewer Dr. Seid Kemal from ICARDA for valuable comments and suggestions. This research was funded by the CRP Dryland Cereals Program. The authors declare that there is no conflict of interest.

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How to cite this article: Gyawali S, Verma RPS, Kumar S, et al. Seedling and adult-plant stage resistance of a world collection of barley genotypes to stripe rust. *J Phytopathol*. 2017;00: 1–10. <https://doi.org/10.1111/jph.12655>