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RESEARCH ARTICLE

Detection of new *Yr1*-virulences in *Puccinia striiformis* f. sp. *tritici* population and its sources of resistance in advance wheat lines and released cultivars

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ABSTRACT: Five hundred and eight yellow rust (YR) samples were pathotyped from Northern states of India during 2014-15 and 2015-16 cropping seasons. The virulence of these samples was tested on 16 wheat differentials and 6 supplementary varieties. Eleven known pathotypes were identified from the analysis of 508 YR samples during this period. Additionally, the infection types of three samples on differentials, collected from US Nagar (Uttarakhand), Kangra and Kinnaur (Himachal Pradesh) districts, were dissimilar to the known pathotypes in India. Subsequently, three isolations were taken from each sample, established separately on susceptible check (A-9-30-1) and each isolation was used to inoculate differentials. All the isolations taken from single sample, showed similar reaction types on differentials. It proved the presence of new pathotype. The new pathotypes (virulences) were designated as 111S68, 79S4 and 79S68. Rust resistance genes *Yr2* (Heines VII), *Yr5*, *Yr9, Yr10, Yr15, Yr24, YrA* were found effective to all these new pathotypes, identified in this study. Evaluation of 135 advance lines and released varieties of bread, durum, dicoccum wheat and triticale indicated that new pathotypes (111S68, 79S4, and 79S68) were less virulent than known pts. 47S103 (T) and 47S102 (K) virulent to *Yr1*. More than 70 per cent evaluated wheat and triticale material was found resistant to moderately resistant to all *Yr1*-virulences. New pathotypes, identified from indigenous wheat, would aid in understanding the evolution mechanism in *Yr1*-virulences of yellow rust pathogen in India for better management of yellow rust.

Keywords: Evolution, pathotype, resistance, stripe rust, wheat

Wheat is the second most important cereal and food crop of India in terms of harvested area, production and consumption. India is the second largest producer (93.50 million tonnes during 2015-16) and consumer (88.55 million tonnes in 2015) of wheat in the world. Wheat production is mainly confined to the Indo-Gangetic plains region and three northern states, namely Uttar Pradesh (35.53%), Punjab (18.96%) and Haryana (13.39%), supply 67 per cent of India's total wheat output (Tripathi and Mishra, 2017). Total demand of wheat in 2025 is forecasted to be in the range of 91.4-101.7 million tons (Ganesh-Kumar *et al*., 2012). The current major challenges facing future wheat production in India are increasing heat stress, dwindling water supplies for irrigation and ever-growing threat of new virulence of diseases such as wheat rusts and leaf blight (Joshi *et al*., 2007). Although many diseases and pests are known to reduce grain yield potential and quality of wheat, the three rusts fungi namely, *Puccinia striiformis* f. sp. *tritici* (*Pst*, Yellow/stripe rust), *Puccinia triticina* (*Pt*, Brown/leaf rust) and *Puccinia graminis* f. sp. *tritici* (*Pgt*, Black/stem rust) have historically caused major crop losses because of their widespread prevalence and airborne. Despite the widespread use of resistant cultivars and fungicides, rusts continue to remain a big challenge in India, bringing back unpleasant memories of pre-green revolution era. This group of diseases continue to remain economically important despite the widespread use of host resistance and fungicides (Singh *et al*., 2016). Yellow rust (YR) can cause 100 per cent yield losses if infection occurs very early and the disease continues developing during the growing season. In most wheat-producing areas, yield losses caused by stripe rust have ranged from 10 to 70 per cent depending on susceptibility of the cultivar, earliness of the initial infection, rate of disease development, and duration of disease (Chen, 2005). YR is more common in cooler areas of Northern India and Nilgiri hills in Southern India. It appeared in epidemic form in the plains of Jammu and Kashmir, foothills of Punjab and Himachal Pradesh and tarai region of Uttarakhand during 2010-11 crop season, but timely action on controlling its spread through fungicide saved the crop from major damage (Bhardwaj *et al*., 2012; Sharma and Saharan, 2011). Occurrence of yellow rust in high severity during the last decade was due to

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evolution of more virulent pathotypes that were able to overcome widely used sources of resistance in wheat (Prashar *et al.*, 2007). Virulence information on *Pst* pathotypes is important to implement effective disease management with resistant cultivars (Sharma-Poudyal *et al.,* 2013). The success of any breeding program to produce rust resistant cultivars depends on knowledge of virulences of a rust pathogen and their infection types on wheat. In this paper, detailed information about newly emerged *Pst* pathotypes virulent to *Yr1* rust resistance gene and sources of resistance in advance wheat material and released varieties are discussed.

MATERIALS AND METHODS

Yellow rust samples collection, analysis and determination of pathotypes

Yellow rust leaf samples collected from infected wheat plants were established on 7-8 day old seedlings of susceptible line A-9-30-1. Fresh uredosporic growth appeared on A-9-30-1 within 15 days after inoculation (DAI). Inoculum (uredospores) was collected at 20th DAI from individual sample on a non-hygroscopic butter paper and was used for inoculating the sets of wheat differentials.

Pathotypes were identified based on infection types (ITs) produced on a group of 16 wheat differentials divided into three sub groups. The first sub group named as set-A (composed of selected world differentials) and the second sub group, designated as set-B included Indian and selected European differentials (Table 1). Set-0, third sub group consisted of Indian wheat cultivars developed recently along with two susceptible checks of wheat and barley which provided supplementary information useful for identification of different pathotypes. The seedlings of stripe rust differentials were grown in aluminium bread pans (29 cm long × 12 cm wide × 7 cm deep size) in a mixture of fine loam and farmyard manure (3:1) that was sterilized by autoclaving at 60°C for an hour. Thirty holes in three rows (3cm depth and 4cm apart, 10 holes in each row) were made with the help of wooden markers and all the constituents of the group were sown in one bread pan. For each differential and elite line only 5-6 seeds were sown in one hole. The inoculation was done on 6-8 days old seedlings by scraping the uredospores from the infected leaf with lancet needle and smearing the scraped inoculum on differential lines. Thereafter, differential sets were sprayed with a fine mist of water and placed in dew chambers (RH>80%) at 12±2°C for 48 h with 12 h of daylight photoperiod. The inoculated material was then removed and put on benches in a greenhouse where temperature of 16±2°C, relative humidity of 60-80% and illumination of about 15,000 lux for 12-16 h photoperiod were maintained.

Infection types of *Pst* pathotypes on wheat differentials were scored at 20 days post- inoculation (DPI), following the method suggested by McIntosh *et al.* (1995). Infection types were characterized as 0 (Immune) = no visible uredia, (Highly Resistant) = Necrotic flecks; N (Resistant) = Necrotic area without sporulation, 1 (Resistant) = Necrotic and chlorotic areas with restricted sporulation, 2 (Moderately resistant) = moderate sporulation with necrosis and chlorosis, 3 (Moderately susceptible) = Sporulation with chlorosis, 3+/4 (Susceptible) = Abundant sporulation without chlorosis. Infection type 33+ was used when both 3 and 3+ pustules occurred together. The experiment was conducted twice to confirm the reaction types. If a *Pst* isolate showed any difference in pathogenicity from the old pathotypes, then, isolation was taken, reaction types confirmed and new pathotype was designated as per the binary notation system (Nagarajan *et al*., 1983) with modification (Bhardwaj *et al*., 2012).

Evaluation of Indian advance wheat lines

A set 135 advance wheat lines (AVT I, 2016-17) and selected released varieties comprising of aestivum, durum, dicoccum wheat and triticale, were evaluated against the three new pathotypes (111S68, 79S68, 79S4) and two known *Yr1*-virulent pts. 47S1103 (T) and 47S102 (K). The list of 135 wheat lines/varieties and their response to *Pst* pathotypes is shown in Table 4.

For evaluating wheat elite lines, only 18 holes were made in one bread pan. Further, the elite lines were inoculated using a glass atomizer containing 10 mg spores of a *Pst* pathotype suspended in 2 ml light grade

Table 1. Set of differentials for the identification of *Pst* pathotypes on wheat in India

Set-A (Selected Word differentials)	*Yr*-gene	Set-B (Selected European and Indian differentials)	*Yr*-gene	Supplemental differential cultivars	*Yr*-gene
Chinese166	*1*	Hybrid46	*3b, 4b*	WH147	-
Lee	*7, 22, 23*	HeinesVII	*2, +*	Bilara 2 (Barley)	-
Heines Kolben	*2, 6*	Compair	*8, 19*	PBW343	*9, 27*
Vilmorin23	*3a, 4a, +*	TSA	*5, +*	HS240	*9, +*
Moro	*10*	Tc*6/Lr26	*9, +*	Anza	*A*
Strubes Dickkopf	*2, 3a#, 4a#, 25*	Sonalika (SK)	*2, A, +*	A-9-30-1	-
Suwon92 × Omar	*Su*	Kalyansona (KS)	*2, +*		
Riebesel47/51	*2, 9, +*	*Yr*24/*Yr*26	*24/26*		

- = no resistance gene known, + additional resistance genes known, # status of these genes not yet known but continue to be mentioned in the literature

Table 2. Distinguishing virulence of new and known *Pst* pathotypes on wheat differentials

Wheat differentials	*Yr*-gene	New and known *Pst* Pathotypes				
		47S102 (K)	111S68*	79S68*	79S4*	47S103 (T)
Strubes Dickkopf	*2, 3a, 4a, 25*	V	V	Av	Av	V
Suwon92 × Omar	*Su*	Av	V	V	V	Av
Hybrid46	*3b, 4b*	Av	Av	Av	Av	V
HeinesVII	*2, +*	V	Av	Av	Av	V
Sonalika (SK)	*2, A, +*	V	Av	Av	Av	V
Kalyansona (KS)	*2, +*	V	V	V	Av	V
Anza	*A*	V	Av	Av	Av	V

* New *Pst* pathotypes identified in this study, Av-avirulent (0; , ; , ;1), V-virulent (2+, 3-, 3+) based on 0-4 scale as described by McIntosh *et al.* (1995)

Table 3. Avirulence /virulence structure of new pathotypes

Pathotypes	Location and year of detection	Avirulence	Virulence
111S68	US Nagar (Uttarakhand), 2015	*Yr2* (Heines VII), *Yr3b, Yr4b* (Hybrid46), *Yr5* (TSA), *Yr9* (Tc*6/*Lr*26), *Yr2*+*Yr9* (Riebesel47/51), *Yr10* (Moro), *Yr24* (or *Yr26*), *YrA* (Sonalika)	*Yr1* (Chinese166), *Yr2* (Kalyansona), *Yr3a, Yr4a* (Vilmorin23), *Yr2, Yr6* (Heines Kolben), *Yr8, Yr19* (Compair), *Yr7, Yr22, Yr23* (Lee), *Yr2, Yr3a, Yr4a, Yr25* (Strubes Dickkopf), *YrSu* (Suwon92 × Omar)
79S4	Kangra, (Himachal Pradesh), 2016	*Yr2* (Heines VII), *Yr3b, Yr4b* (Hybrid46), *Yr2, Yr3a, Yr4a, Yr25* (Strubes Dickkopf), *Yr2* (Kalyansona), *Yr5* (TSA), *Yr9* (Tc*6/*Lr*26), *Yr2*+*Yr9* (Riebesel47/51), *Yr10* (Moro), *Yr24* (or *Yr26*), *YrA* (Sonalika)	*Yr1* (Chinese166), *Yr3a, Yr4a* (Vilmorin23), *Yr2, Yr6* (Heines Kolben), *Yr8, Yr19* (Compair), *Yr7, Yr22, Yr23* (Lee), *YrSu* (Suwon92 × Omar)
79S68	Kinnaur, (Himachal Pradesh), 2016	*Yr2* (Heines VII), *Yr3b, Yr4b* (Hybrid46), *Yr2, Yr3a, Yr4a, Yr25* (Strubes Dickkopf), *Yr5* (TSA), *Yr9* (Tc*6/*Lr*26), *Yr2*+*Yr9* (Riebesel47/51), *Yr10* (Moro), *Yr24* (or *Yr26*), *YrA* (Sonalika)	*Yr1* (Chinese166), *Yr2* (Kalyansona), *Yr3a, Yr4a* (Vilmorin23), *Yr2, Yr6* (Heines Kolben), *Yr8, Yr19* (Compair), *Yr7, Yr22, Yr23* (Lee), *YrSu* (Suwon92 × Omar)

mineral oil (Soltrol 170®) (Chevron Phillips Chemicals Asia Pvt. Ltd., Singapore). After inoculation, the oil was allowed to evaporate for 10 min. Remaining study was conducted as described above for differentials.

RESULTS

Phenotypic characterization of new pathotypes

During 2014-15 cropping season, 295 yellow rust samples were pathotyped on wheat differentials. A sample (*Pst*-254/36.2015) collected from infected leaves of local wheat variety grown at farmer's field located in US Nagar district of Uttarakhand, showed reaction types different from known *Yr1*-virulences. Isolations were taken, one each from chinese166, Strubes Dickkopf and Suwon92 × Omar and were multiplied on susceptible check (A-9-30-1). Subsequently, each isolation was used separately to inoculate the sets of differentials and compared with closely related *Yr1*-virulences 47S102 and 47S103. The test isolations showed similar kind of reactions but different from known pathotypes (Table 2). The test isolation possessed the combined virulence for Strubes Dickkopf and Suwon92 × Omar. This new *Yr1* virulence was designated as 111S68. The avirulence/ virulence formula is described in Table 3.

In total, 213 YR samples were characterised on wheat differentials during 2015-16 cropping season. A sample (*Pst*-162/10.2016) was collected from wheat leaves with uredospores from Kangra district of Himachal Pradesh, showed reactions on differentials which were distinct from known pathotypes virulent to *Yr1*. Isolations taken from differentials, like the earlier one was first established on susceptible host, and then compared with closely related known pts. 47S102 and 47S103. All Isolations taken from YR sample (*Pst*-162/10.2016) showed similar reactions on differentials but distinct from the known ones. It proved that this was a new pathotype and designated as 79S4.

Similarly, another sample (*Pst*-01/10.2016) which was collected from Kinnaur district of Himachal Pradesh, possessed virulence to *Yr1* but different from known ones, was designated as 79S68. The avirulence/virulence formula is described in Table 3.

New pt.111S68 is different to the known pt.47S103 (T) in having additional virulence for Suwon92 × Omar and lacking virulence for Hybrid46, Heines VII and Sonalika (*YrA*). This new *Pst* pathotype possesses combined virulence to Strubes Dickkopf and Suwon92 × Omar. The pts. 79S68 and 79S4 are distinguishable on Kalyansona (*Yr2*) as shown in Table 2. The former

Table 4. Response of advance wheat lines (AVT I, 2016-17) and released varieties to new and known *Yr*-virulences of *Puccinia striiformis* f. sp. *tritici*

Advance wheat lines and released varieties	47S102 (K)	79S68*	79S4*	111S68*	47S103 (T)
AKDW2997-16(D)#	MS	R	R	MS	S
BRW3773	S	R	R	MS	S
BRW3775	MS	R	R	R	MS
C306#	R	MS	R	R	S
CG1023	S	MS	R	MR	R
DBW14#	R	R	R	MR	R
DBW39#	R	R	R	R	R
DBW71#	R	R	R	R	R
DBW88#	R	R	R	R	R
DBW90#	R	R	R	R	S
DBW110#	R	R	R	R	MS
DBW168	S	R	R	R	R
DBW173	R	R	R	R	R
DBW179	R	R	R	R	S
DBW187	S	R	R	R	MS
DBW189	R	MS	R	R	R
DBW196	S	S	R	R	S
DBW246	R	R	R	R	R
DBW247	S	MR	R	MS	S
DBW248	MR	R	R	R	R
DBW249	R	R	R	MS	MS
DBW250	R	R	R	MS	S
DBW251	R	R	R	R	S
DDK1029 (Dic)#	MS	S	R	S	MS
DDK1052 (Dic)	MS	MS	R	R	R
DDK1053 (Dic)	S	S	R	S	MS
GW322#	S	R	R	MS	S
HD2733#	R	R	R	R	R
HD2828#	R	R	R	R	S
HD2967#	S	R	R	R	S
HD3043#	R	R	R	R	R
HD3059#	R	R	R	R	MR
HD3086#	S	R	R	R	S
HD3171#	S	R	R	R	R
HD3219	MS	R	S	S	MS
HD3226	MS	R	R	R	R
HD3237	S	R	R	MS	MS
HD3271	R	R	R	R	R
HD3272	MR	R	R	R	S
HI1612	R	R	R	R	S
HI1617	R	R	R	R	R
HI1619	S	R	R	R	S
HI1620	S	R	R	R	S
HI1621	S	MS	MS	MS	S
HI8627 (D) #	R	R	R	R	R
HI8777 (D)	S	R	R	R	R
HI8791(D)	R	R	R	R	R
HP1963	S	R	R	R	S

Contd...

HPW251#	R	R	R	R	R
HPW439	R	R	R	R	S
HPW440	R	MR	R	R	MS
HPW448	R	R	R	R	R
HPW449	R	R	R	R	R
HS375#	R	R	R	R	R
HS490#	R	R	R	R	R
HS507#	R	R	R	R	R
HS542#	R	R	R	R	MS
HS611	S	R	R	MS	S
HS629	MS	R	R	S	R
HS630	S	R	R	MS	S
HS643	S	MS	R	S	S
HS644	R	R	R	R	R
HS645	R	R	R	R	R
HS646	R	R	R	R	R
HS647	R	R	R	R	R
HS648	MS	R	R	R	S
HW1098#	MS	S	R	MS	MS
HW2044#	R	R	R	R	R
HW5216#	R	R	R	R	R
K0307#	S	R	R	MS	S
K1006#	S	R	R	S	S
K1317#	S	R	R	S	MS
K8027#	S	R	R	R	MS
Kharchia 65#	S	S	S	S	S
KRL19#	S	S	R	S	S
KRL210#	S	R	R	R	S
KRL370	S	R	R	MS	S
KRL377	MS	R	R	R	MS
KRL384	S	R	R	R	R
KRL386	S	R	R	MS	S
MACS4028 (D)	S	S	MS	S	S
MACS5047	S	MS	R	MS	MS
MACS5049	MR	R	R	MS	MS
MACS6222#	R	R	R	MS	MS
MACS6478#	MS	R	R	MS	MS
MACS6677	S	R	R	R	S
MP1318	R	R	R	R	R
MP3288#	MS	R	R	R	MS
NI5439#	S	S	S	S	S
NIAW1415#	R	R	R	R	R
PBW550#	R	R	R	R	R
PBW644#	R	R	R	MS	R
PBW750	R	R	R	R	R
PBW752	R	R	R	R	R
PBW757	R	R	R	R	R
PBW777	R	R	R	R	R
PBW778	MS	MR	R	R	S
PBW779	R	R	R	R	R
PBW780	R	R	R	R	R
TL2942#	R	R	R	R	R

Contd...

TL2969#	R	R	R	R	R
TL3011	R	R	R	R	R
TL3012	R	R	R	R	R
TL3013	R	R	R	R	R
TL3014	R	R	R	R	R
TL3015	R	R	R	R	R
UAS304#	S	MS	S	S	S
UAS375	R	R	R	R	R
UAS384	S	R	R	S	R
UAS385	S	R	R	MS	S
UAS387	R	R	R	R	R
UAS446#	MS	S	R	MS	MS
UAS462 (D)	R	MS	S	S	S
UP2942	R	R	R	R	R
UP2992	MS	R	R	R	MS
UP2993	R	R	R	R	R
VL829#	R	R	R	R	R
VL892#	R	R	R	R	R
VL1011	MS	R	R	R	R
VL1012	R	R	R	R	R
VL1013	R	R	R	R	R
VL3013	S	R	R	R	R
VL3014	S	R	R	R	MS
VL3015	R	R	R	R	R
VL4003	R	R	R	R	R
WH1021#	R	R	R	R	R
WH1080#	S	R	R	MS	S
WH1105#	R	R	R	R	MS
WH1124#	MS	R	R	R	S
WH1142#	R	R	R	R	R
WH1202	R	R	R	R	R
WH1232	R	R	R	R	S
WH1233	R	R	R	R	R
WH1316	MS	S	R	S	MS
WR544#	S	R	R	MS	S

D = Durum wheat, Dic = Dicoccum wheat, * New pathotypes, # released varieties

pathotype is virulent on Kalyansona (*Yr2*), whereas, the latter one is avirulent.

Evaluation of advance wheat lines and released varieties

In total, 135 advance lines and released varieties of bread, durum, dicoccum wheat and Triticale were screened for rust resistance against new (79S68, 79S4 and 111S68) and known (47S102 and 47S103) pathotypes of yellow rust pathogen (Table 4). In this study, 53 (39.3%) lines/varieties were found immune (IT: 0;, ; or ;-) and only two genotype, Kharchia65 and NI5439, highly susceptible (IT: 3+, 33+) to all pathotypes possessed virulence to *Yr1*. Among 135 advance lines/ released varieties, 92 were found resistant to moderately resistant (IT: 0;, ;, ; 1, 2-) to new pts. 79S68, 79S4 and 111S68. Advance line DBW189 and a released variety PBW644 which were resistant to both known pathotypes (47S103 and 46S102), showed moderately susceptibility to new pts. 79S68 and 111S68, respectively. On the other hand, BRW3775, DBW187, HD2967, HD3086, HI1619, HI1620, HI1963, HS648, K8027, KRL210, KRL377, MACS6677, MP3288, PBW778, UP2992, VL3014 and WH1124 were resistant to new pathotypes (79S68, 79S4 and 111S68) but found susceptible to moderately susceptible to known pathotypes (T and K). In toto, 74, 113, 128, 95 and 68 advance lines/ released varieties were resistant, 17, 9, 2, 23 and 24 lines moderately susceptible, 41, 10, 5, 15 and 42 lines susceptible to pts. 47S102 (K), 79S68, 79S4, 111S68 and 47S103 (T), respectively (Fig. 1).

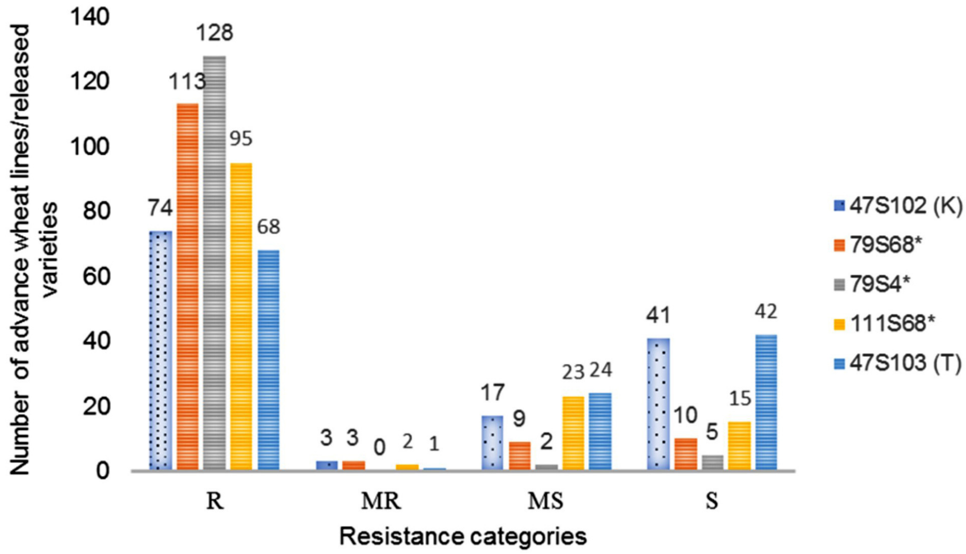

Fig. 1. Number of advance lines and released varieties of bread, durum, dicoccum wheat and Triticale in different resistance categories to new and known *Yr1*-virulent pathotypes of yellow rust pathogen

DISCUSSION

Deploying the rust resistance genes in wheat cultivars remains a sustainable approach to manage this devastating wheat rust diseases. However, new pathotypes/races evolved quickly rendering previously resistant cultivars susceptible (Wan and Chen, 2012; Bhardwaj *et al*., 2016). In India, first virulence to *Yr1* was detected in 1936 and designated as 31 (67S64). Subsequently, pt. 13 (67S8) in 1937, K (47S102) in 1982, T (47S103) in 1992, CII (15S64) in 1993 and 7S0 in 2012, were identified and confirmed based on reaction types on wheat differentials (Nayar *et al*., 1997; Gangwar *et al*., 2016). In Indian *Pst* population, many virulences have been detected on *Yr1* even though there is no evidence of its use in wheat breeding programme. Such mutation, unrelated to host resistance genes, have been observed earlier and were attributed to their associations with better survival/adaptation of the pathogen. Authors of this study also felt that these mutations have provided yellow rust pathogen with an advantage. Mutation coupled with the directional selection, somatic and sexual recombination are considered to be mechanisms determining the genetic variability of *Puccinia striiformis* f. sp. *tritici* that result in the evolution of new aggressive races or pathotypes and breakdown of major resistance gene (Hovmoller *et al*., 2011; Jin, 2011; Park and Wellings, 2012; McDonald and Linde, 2002). In present study, three new *Yr1* virulences of yellow rust pathogen which were identified in the year 2015 (pt. 111S68) and 2016 (79S4, 79S68), also presumed to have evolved by the mechanism of mutation. The virulence spectra of these pathotypes is narrower than earlier known *Yr1*-virulences (T and K) as is evident from data on differentials and wheat genotypes. However, their geographical distribution is wider than previously identified *Yr1*-virulent pt. 15S64 (CII) which is less virulent and confined to Leh-Ladakh regions of Jammu and Kashmir (Bhardwaj *et al*., 2012). Although, more than 70 per cent wheat advance lines and released varieties retained resistance to these pathotypes but a significant portion of the evaluated lines were found susceptible to new pathotypes. New pathotypes have been added to national repository of pathotypes of rust pathogens at ICAR-IIWBR, Shimla, Himachal Pradesh.

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