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Physiologic specialization and new virulences of *Puccinia graminis* f sp *tritici* causing black rust of wheat *(Triticum aestivum)* in India during 2005-2009

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ABSTRACT

During 2005-09, black (stem) rust of wheat (*Triticum aestivum* L.) was observed mainly in the Nilgiri hills (Tamil Nadu) and in Karnataka. Low incidence was also observed in Madhya Pradesh, Maharashtra and Gujarat, whereas black rust was observed in North Indian hills in Almora (Uttarakhand) and in summer crop in Himachal Pradesh in 2007. More than 300 samples of black rust of wheat were analyzed mainly from Nilgiri hills (Tamil Nadu) and Karnataka. Pathotypes 62G29 (40A) with virulence to *Sr*2, *Sr*5, *Sr*7b, *Sr*8a, *Sr*8b, *Sr*9b, *Sr*9e, *Sr*11, *Sr*28 and 62G29-1(40-1) similar to 40A but with additional virulence to *Sr*24 were predominant in Nilgiri hills. The frequency of pathoype 62G29-1 (virulent on *Sr24*) showed increasing trend in Tamil Nadu. Predominant pathotypes 166G2(117-1), 167G3(117-3) and 58G13-3(40-2) in Karnataka appear to be especially virulent on durum and dicoccum wheat. Four new pathotypes were identified during this period including virulence for *Sr*25 which was resistant till now. New pathotype from Maharashra is virulent to *Sr*5, *Sr*8a, *Sr*9b, *Sr*9e and *Sr*11. Three pathotypes, virulent to *Sr5, Sr*8a, *Sr*9b, *Sr*9e, *Sr*28 and *Sr*30 were identified from Karnataka where other *Sr* genes namely, *Sr*11 and *Sr*36 were also affected. Black rust resistance genes *Sr26, Sr27, Sr*31, *Sr*32, *Sr*33, *Sr*35, *Sr*39, *Sr*43 were effective and are resistant to all the pathotypes of black rust in India.

Key words: Pathotype identification, Stem rust, *Sr* genes, Wheat.

Black (stem) rust caused by *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & Henn., historically is one of the most destructive diseases of wheat (*Triticum aestivum* L.) that has again threatened the wheat production worldwide due to the emergence and spread of race Ug99 (TTKSK) and its variants having virulence for *Sr*31*, Sr*24 and *Sr*36 (Pretorius *et al.* 2000, Singh *et al.* 2011). The dynamic nature of black rust pathogen provides a continuous threat to wheat varieties in use.The incidence and virulence pattern of black rust pathogen are monitored on wheat crop in India for early detection of possible new virulence, evolution of pathotypes and, changes in pathotype composition and their distribution patterns on summer and regular wheat crop. The information, thus, generated is used to select black rust resistance genes to be incorporated in developing new wheat varieties to diversify resistance for avoiding heavy losses. This communication summarizes incidence and pathotypes of *P. graminis tritici* prevalent in India and emergence of new virulences during 2004-05 to 2008-09 crop seasons.

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MATERIALS AND METHODS

During 2005 to 2009 crop seasons, black rust infected stem and leaf samples of wheat were received/collected from nurseries and farmers' fields from different states of India including from summer/off-season crops and self-sown plants. These samples were established by inoculating primary leaves of 7-day-old seedlings of Agra local (a susceptible bread wheat) in glasshouse. Inoculated pots were kept in humid chamber after spraying with the fine mist of water for 48 hr and then kept out on the glasshouse benches at 24±2°C temperature with supplemental light if needed. Fresh uredosporic inoculum was used for further studies.

Fresh uredospores of each samples developed on leaves were used to inoculate the sets of differential lines for identification of pathotype of black rust (Nayar *et al*. 1997). Infection types (ITs) on the differential lines were recorded 15-17 days post inoculation following Stakman *et al.* (1962). Infection types were categorized into resistant (0, 0; (fleck),:1, 1, 2 and 2+) and susceptible (3, 33+ and 3+).

In India, the black rust pathotype designation system (Bahadur *et al*. 1985, Nayar *et al*. 1997) is based on binary notation system and comprised of three sets, Set-A, Set-B and Set-0. In this, a fixed exponential value is given to every differential line. Black rust ITs on these lines are scored as

resistant (binary score=0) and susceptible (binary score=1). The binary score was multiplied with the decanery value and a value thus obtained for each differential line. These values are added for Set-A and Set-B separately which becomes the number for each Set. The sum of each set is separated by a letter G (for *Puccinia graminis tritici*) that gives the designation of the pathotype. Set-0 mostly comprised of predominantly cultivated wheat varieties in India, resistant line and susceptible cultivars of wheat to know the response of these varieties/lines against prevalent/new pathotypes. If a previously resistant cultivar becomes susceptible to a new pathotype, the serial number of that particular line is added to designate the pathotype. New pathotypes were also designated as per the North American nomenclature system to know the international equivalent (Roelfs and Martens 1988, Jin *et al.* 2008).

Whenever infection types on differential sets appeared to be different from the known or existing pathotypes then, subsequently, 4-5 single pustule isolations were taken and tested on differential lines. The putative new pathotypes were evaluated simultaneously with known pathotypes on sets of differential lines and confirmed with repetitive testing. The virulence phenotype of the new pathotype was determined by evaluating near-isogenic lines of *Sr* genes and lines carrying specific *Sr* genes at seedling stage at 2 different temperatures (16°C, 25°C) in different glasshouses. These evaluations were repeated for the consistency of the results.

Based on the avirulence/virulence formulae of the pathotypes identified during the period and infection types recorded on near-isogenic lines and differential lines, effectiveness or ineffectiveness of common *Sr* genes found in Indian wheat materials was estimated. Pathotype distribution and *Sr* genes effectiveness was analyzed using descriptive statistics (frequencies and percentages).

RESULTS AND DISCUSSION

Incidence of black rust and pathotype distribution

Occurrence of wheat black rust in India was not widespread and severe during 2004-05 to 2008-09 crop seasons. It was restricted to only few states and the incidence was also variable in different years as revealed by the samples analysis data from different states (Table 1). Black rust is regularly occurring in the Nilgiri hills in Tamil Nadu where wheat is grown throughout the year in experimental station and samples were analyzed in all the five years. Black rust was also reported from Karnataka, Maharashtra, Gujarat and Madhya Pradesh in 3 / 4 years out of five years reported here but the sample analyzed were less in comparison to Tamil Nadu (Table 1). Due to shift for cultivation of cash crops mainly vegetables, wheat cultivation in Tamil Nadu is restricted mainly to some areas only. Majority of wheat black rust samples from Tamil Nadu were from Regional Station of IARI, Wellington where wheat is grown around the year. Only in 2007-08 from Karnataka, more samples were analyzed as black rust was observed in many locations at Arabhavi, Ugar and adjoining areas mainly on durum and dicoccum wheats. Apart from these states, late infection of black rust was also observed in Alomra in Uttarakhand (northern Himalyan hills) in 2007 on few plants when wheat crop was nearly matured (in April). Similarly, black rust was also observed in summer wheat crop in August 2007 at Tabo (Lahaul & Spiti) in Himachal Pradesh but the incidence was low. The low incidence of black rust in these two Himalayan states may be because of stem rust pathogen requires warm temperatures to thrive, thus, there was lack of sufficient inoculum multiplication in these cooler regions.

Three hundred twenty two isolates from black rust samples were analyzed and pathotypes identified from six states during these five years. Eleven pathotypes including four new ones were identified from these samples (Table 1). The most frequent pathotype was 62G29 (45%) followed by 62G29-1 (24.8%), 166G2 (15.2%) and a new pathotype 58G13-3 (6.5%). In Tamil Nadu, 4 pathotypes were observed, whereas in Karnataka seven pathotypes were identified.

Pathotype 62G29 (40A) was the most widespread pathotype of *P. graminis* f. sp. *tritici* identified in wheat samples from almost all the states (Table 1,2). This pathotype has been the most predominant in the black rust population in India for the last 30 years (Bhardwaj 2012) and is virulent to *Sr*5, *Sr*7b, *Sr*8a, *Sr*9b, *Sr*9e, *Sr*11, *Sr*28 and on Charter (*Sr*11+). Pathotype 62G29-1 (40-1), similar to 62G29 but with additional virulence for *Sr*24, remained restricted in the Nilgiri hills in Tamil Nadu since first identified in 1989 (Bhardwaj *et al*.1990), was also picked up in 80 samples in 5 years. Though, pathotype 62G29 has remained predominant for many years but the population of *Sr*24-virulent pathoype (62G29-1) showed increasing trend during these years and identified in more than 50% of the samples in 2008 and 2009 in Tamil Nadu (Table 1, Fig 1). Pathotype 7G11 (122) was also found in 3 samples from Nilgiri hills.

In Karnataka, a new pathotype virulent on *Sr*25 (58G13-3) was identified for the first time in India in 2005-06 and was found in 16 out of the 20 samples analyzed and further

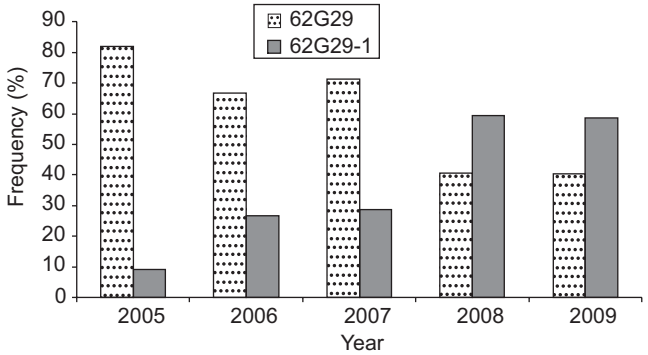

Fig 1 Frequency of pathotypes 62G29 and 62G29-1 (virulent on *Sr*24) in Nilgiri hills

Table 1 Pathotype distribution of *Puccinia graminis tritici* during 2005-09 in India

State	Year/Season	No. of isolates analyzed	Existing pathotypes					New pathotypes				
			62 G 29	62 G 29-1	37 G 19	166 G 2	167 G 3	7 G 11	55 G 1	58 G 13-3	123 G 15	127 G 29
Tamil Nadu	2004-05	10	9	1								
	2005-06	30	20	8				2				
	2006-07	14	10	4								
	2007-08	32	13	19								
	2008-09	82	33	48				1				
Karnataka	2005-06	20	4							16		
	2006-07	2								2		
	2007-08	77	7			49	16			3	1	1
Maharashtra	2004-05	7	6					1				
	2005-06	1							1			
	2006-07	11	11									
	2008-09	5	5									
Gujarat	2004-05	12	12									
	2005-06	2	2									
	2007-08	1	1									
Madhya	2004-05	9	9									
Pradesh	2006-07	4	2		2							
	2008-09	2	1		1							
Total		322*	145	80	3	49	16	4	1	21	1	1

*One sample from summer crop in Himachal Pradesh in 2007 yielded pathotype 10G13 virulent on *Sr*24.

identified in a few samples in following years (Table 1). This pathotype is virulent on *Sr*5, *Sr*7b, *Sr*9b, *Sr*9e, *Sr*13, *Sr*14, *Sr*25, *Sr*28, *Sr*30 but avirulent on *Sr*11 (Jain *et al*. 2009). In 2007-08, as many as 76 samples were analyzed from Karnataka where pathotype 166G2 (117-1) was the most predominant, found in 49 samples followed by 167G3 (117-3). These two pathotypes are particularly virulent on durum and diccocum wheat and are virulent to *Sr*9e, *Sr*11 and *Sr*21. In the same year, one sample of black rust yielded two new pathotypes designated as 123G15 and 127G29.

In Maharashtra, pathotype 62G29 was identified in most of the samples, however, single sample analyzed in 2004-05 recorded a new pathotype 55G1 (184-1) which is virulent to *Sr*7b, *Sr*8a, *Sr*9b, *Sr*9e, *Sr*11 and *Sr*13 (Jain *et al.* 2011). In Gujarat and Madhya Pradesh, 15 samples each were analyzed in 3 years where pathotype 62G29 was identified in all the samples except 3 samples from Madhya Pradesh that yielded pathotype 37G19 (117-6) virulent to *Sr*9e, *Sr*11, *Sr*13, *Sr*21 and avirulent to *Sr*5, *Sr*9b, *Sr*28. Overall, *Sr*25 virulence is important and needs vigilance. Frequency of virulence to *Sr*5, *Sr*7b, *Sr*9b, *Sr*9e, *Sr*11, *Sr*30 and *Sr*36 is increasing. Virulence to *Sr*9e is very common in central and southern part of the country where durum and diccocum wheats are grown.

Effectiveness of Sr genes

A perusal of Table 3 indicates that the *Sr26, Sr27, Sr*31,

Table 2 Ineffective genes of eleven pathotypes identified during 2005-09

Pathotypes identified		Most common ineffective/ susceptible *Sr* genes	No. of isolates
Binary notation system	North** American equivalent		
10G13 (34-1)	MHGSF	*Sr*5, *Sr*7b, *Sr*9b, *Sr*24, *Sr*28	01
62G29 (40A)	PTHSS	*Sr*5, *Sr*7b, *Sr*8a, *Sr*9b, *Sr*9e, *Sr*11, *Sr*28	145
62G29-1 (40-1)	PTHSH	*Sr*5, *Sr*7b, *Sr*8a, *Sr*9b, *Sr9e*, *Sr*11, *Sr*24, *Sr*28	80
166G2 (117-1)	JRHSC	*Sr*9b, *Sr*9e, *Sr*11, *Sr*21, *Sr*37	49
167G3 (117-2)	KRHSC	*Sr*7b, *Sr*9b, *Sr*9e, *Sr*11, *Sr*13, *Sr*21, *Sr*37	16
37G19 (117-6)	KRHSC	*Sr*7b, *Sr*9e, *Sr*11, *Sr*13, *Sr*21	03
7G11 (122)	RRHSC	*Sr*7b, *Sr*9b, *Sr*11, *Sr*13, *Sr*21	04
New pathotype			
55G1 (184-1)	FTHSC	*Sr*7b, *Sr*8a, *Sr*9b, *Sr*9e, *Sr*11, *Sr*13	01
58G13-3 (40-2)	PKTSC	*Sr*5, *Sr*7b, *Sr*8a, *Sr*9b, *Sr*9e, *Sr*25, *Sr*28, *Sr*30	21
123G15 (15-1)	TKTSF	*Sr*5, *Sr*7b, *Sr*8a, *Sr*9b, *Sr*9e, *Sr*21, *Sr*28, *Sr*30	01
127G29 (40-3)	PTTSF	*Sr*5, *Sr*7b, *Sr*8a, *Sr*9b, *Sr*9e, *Sr*11, *Sr*28, *Sr*30	01

*Ineffective *Sr* genes denotes susceptible reactions (3, 33+, 3+ infection types), **North American equivalents (Jin *et al.* 2008)

Table 3 Percent effectiveness of *Sr* genes against black rust population

State	*Sr* genes*								
	*Sr*5	*Sr*8a	*Sr*9b	*Sr*9e	*Sr*13	*Sr*21	*Sr*24	*Sr*30	*Sr*37
Tamil Nadu	0	1.7	0	1.7	98.3	98.3	52.4	100	100
Karnataka	65.3	65.3	0	0	11.2	12.2	100	76.5	43.8
Maharashtra	0	4.1	2.1	2.1	95.8	100	100	100	100
Gujarat	0	0	0	0	100	100	100	100	100
Madhya Pradesh	2	2	2	0	92	92	100	100	100

* *Sr*26, *Sr*27, *Sr31*, *Sr*32, *Sr*33, *Sr*35, *Sr*39, *Sr*43 were effective to all the pathotypes

*Sr*32, *Sr*33, *Sr*35, *Sr*39, *Sr*43 genes were effective and are resistant to all the pathotypes of black rust in India. During 2005-09, *Sr*30 and *Sr*37 genes were found effective (100%) in all states except in Karnataka where pathotypes virulent on these genes including new ones were recorded. Similarly, *Sr*24 gene was effective in most of the states except in Tamil Nadu where pathotype 62G29-1 (*Sr*24 virulent) has shown increasing trend in its frequency. *Sr*13 and *Sr*21, the two genes which are found in durum and dicoccum wheat, were found highly effective in every states except in Karnataka where 117 group of pathotypes rendering these genes ineffective are predominant.Other common genes like *Sr*5, *Sr*7b, *Sr*8a, *Sr*9b, *Sr*9e and *Sr*11 have shown very low frequency of effectiveness.

Characterization of new pathotypes and their virulence phenotype

During 2004-09 crop seasons, four new black rust pathotypes were identified. Three new pathotypes were identified from Karnataka and one from Maharashtra. Pathotype 55G1 (184-1), first identified in 2005 from Maharashtra has virulence to *Sr*7b, *Sr*8a, *Sr*9b, *Sr*9e, *Sr*11, *Sr*13 and another new pathotype 58G13-3 (40-2) virulent on *Sr*25, identified from Karnataka in 2006 described in detail in earlier publications (Jain *et al*. 2009, 2011). Two more new pathotypes identified from Karnataka from a single black rust sample received in 2007-08 are described here in detail.

On differential sets, the infection types of this particular sample were different to the existing pathotypes. Many single pustule isolations were taken from differential lines, multiplied and put on differential sets separately. On analysis, two new pathotypes were identified in these isolations, which produced unique infection types different to recorded pathotypes in India. Based on the reactions on differential sets, these pathotypes were designated as 123G15 and 127G29, respectively (Bahadur *et al*. 1985, Nayar *et al*. 1997) following the binary designation system (Table 4).

One isolate was virulent on *Sr*13, *Sr*9b, *Sr*28, *Sr*8a, Vernstein (*Sr*9e) and Festiguay (*Sr*30) in Set A, whereas in set B it was virulent to Marquis (*Sr*7b+), Einkorn (*Sr*21), Kota (*Sr*28+) and Reliance (*Sr*5+), but was avirulent to *Sr*11 and Charter (*Sr*11+). Therefore, this pathotype was designated

Table 4 Black rust infection types on differential sets A and B for the designation of new pathotypes of *Puccinia graminis tritici*

Set/Line	New pathotype	
	123G15 (15-1)	127G29 (40-3)
A-Set		
*Sr*13 (Khapstein/10 Marquis)	3+	3+
*Sr*9b (CS/KF 2B)	3+	3+
*Sr*11 (ISr11Ra= W 3015)	1	3+
*Sr*28 (Kota ;)	3+	3+
*Sr*8a (ISr8aRa= W3384)	3+	3+
*Sr*9e (Vernstein)	3+	3+
*Sr*30 (Festiguay)	33+	33+
*Sr*37 (Line W)	2, X	2-, 2
B-Set		
Marquis (*Sr*7b,18,19,20)	3+	3+
Einkorn (*Sr*21)	X+, 33+	;1
Kota (*Sr*7b,18,28)	3+	3+
Reliance (*Sr*5,16,18,20)		3+ 3+
Charter (*Sr*11+)	;1-	3+
Khapli (*Sr*7a,13,14)	;1	2
Agra local (Susceptible check)	3+	3+

Infection types ;1-, ;1, 2-, 2, x = resistant and 33+, 3+ = susceptible

as 123G15 (Table 4). Based on these infection types, it was found to close to the physiologic race 15 (Stakman *et al.* 1962), which was detected in 1935 in India (Mehta 1941). The new pathotype differs from race 15 (58G15) in having virulence to *Sr*13 and *Sr*30. According to North American system (Jin *et al*. 2008), pathotype 123G15 is designated as TKTSF. This pathotype showed temperature sensitive reactions on Einkorn (*Sr*21) and also on *Triticum monocoocum* line carrying *Sr*21. At 25^0C temperature, infection type (IT) X+ and 33+ were recorded on Einkorn, whereas at lower temperature (16^0C) it gave IT 2. Similarly, on *T. monococcum* line, the reactions were X+3/3 and 3-/3, respectively.

The other new isolate was virulent on *Sr*13, *Sr*9b, *Sr*11, *Sr*28, *Sr*8a, Vernstein (*Sr*9e) and Festiguay (*Sr*30) in Set A whereas in set B it was virulent on Marquis (*Sr*7b+), Kota

Table 5 Avirulence/virulence formula of the two new pathotypes 123G15 (15-1) and 127G29 (40-3)

Pathotype	Avirulent to *Sr* genes	Virulent to *Sr* genes
123G15 (15-1)	*Sr*7a, *Sr*11, *Sr*24, *Sr*25, *Sr*26, *Sr*27, *Sr*31, *Sr*32, *Sr*33, *Sr*35, *Sr*37, *Sr*39, *Sr*40, *Sr*43 *Sr*Gt, *Sr*Tmp, *Sr*Tt3	*Sr*2, *Sr*5, *Sr*6, *Sr*7b, *Sr*8a, *Sr*8b, *Sr*9a, *Sr*9b, *Sr*9d, *Sr*9e, *Sr*9f, *Sr*9g, *Sr*10, *Sr*12, *Sr*13, *Sr*14, *Sr*15, *Sr*16, *Sr*17, *Sr*18, *Sr*19, *Sr*20, *Sr*21, *Sr*22, *Sr*23, *Sr*28, *Sr*29, *Sr*30, *Sr*34, *Sr*36, *Sr*38, *Sr*42, *Sr*44, *Sr*McN, *Sr*Wld
127G29 (40-3)	*Sr*21, *Sr*22, *Sr*24, *Sr*25, *Sr*26, *Sr*27, *Sr*31, *Sr*32, *Sr*33, *Sr*35, *Sr*36, *Sr*37, *Sr*39, *Sr*40, *Sr*42, *Sr*43, *Sr*Tmp, *Sr*Tt3	*Sr*2, *Sr*5, *Sr*6, *Sr*7a, *Sr*7b, *Sr*8a, *Sr*8b, *Sr*9a, *Sr*9b, *Sr*9d, *Sr*9e, *Sr*9f, *Sr*9g, *Sr*10, *Sr*11, *Sr*14, *Sr*15, *Sr*16, *Sr*17, *Sr*18, *Sr*19, *Sr*20, *Sr*23, *Sr*28, *Sr*29, *Sr*30, *Sr*34, *Sr*38, *Sr*44, *Sr* McN, *Sr*Gt

(*Sr*28+), Reliance (*Sr*5+) and Charter (*Sr*11+), but was avirulent on Einkorn (*Sr*21). Thus, this pathotype was designated as 127G29 (Table 4). Since, pathotype 127G29 was avirulent on Einkorn, it was close to the physiologic race 40 (Stakman *et al*. 1962) and chronologically designated as 40-3. This pathotype differs from the most prevalent pathotype in India 62G29 (40A) in having additional virulence for *Sr*13 and Festiguay (*Sr*30) in differential sets. According to North American system (Jin *et al.* 2008), pathotype 127G29 (40-3) is designated as PTTSF.

These new pathotypes have been added to the national repository at Flowerdale. The virulence phenotypes of these pathotypes were determined by the infection types observed on near-isogenic lines of *Sr* genes and lines carrying specific *Sr* genes at seedling stage. The avirulence/virulence formula of pathotypes 123G15 (15-1) and 127G29 (40-3) are given in Table 5. Pathotype 123G15 is also virulent on some of the important resistance genes like *Sr*22, *Sr*36, *Sr*Wld, whereas pathotype 127G29 is avirulent on these genes. However, both the pathotypes are avirulent on *Sr*24 and *Sr*25. Additionally, *Sr* genes resistant to all the pathotypes of black rust in India namely, *Sr*26, *Sr*27, *Sr*31, *Sr*32, *Sr*33, *Sr*35, *Sr*39, *Sr*43, *Sr*Tt3 maintained their resistance to these new virulences also. Hence, the varieties carrying *Sr*31 and *Sr*24, which represent the most commonly grown varieties in India at present, are resistant to these new pathotypes also.

As observed earlier, black rust was largely confined to southern hills, central and peninsular India. Eleven pathotypes were observed in these surveys, four of them new, whereas nine pathotypes were observed in earlier surveys (Bhardwaj *et al*. 2006). The pathotype scenario of *P. graminis tritici* in Karnataka is different to that of Tamil Nadu and is being studied in detail. Pathotype 62G29 (40A) has remained dominant not only in Nilgiri hills but also in all other states. This pathotype appears to have a fitness gene which is linked to virulence making it adaptable for such a long period of more than 30 years (Bhardwaj 2012). Ismail *et al.* (2012) have found that the number of wheat stem rust races identified in a place is not related to wheat stem rust severity in that place. Present study has also found that only 4 pathotypes were observed in Tamil Nadu where black rust occurred regularly whereas 7 pathotypes were observed in Karnataka where black rust was observed only in 3 out of 5 years. The efficacy of common *Sr* genes also remained nearly the same as observed by Bhardwaj *et al*. (2006).

Despite the low incidence in India, the black rust pathogen continues to evolve giving new variations with four new pathotypes have been detected during these 5 years. Thus, evolution of new pathotypes in India and also within Ug99 race in the world warrants continuous vigil on black rust pathogen and consequent search for effective sources of resistance. Identification of new pathotypes in initial stages will help breeders to develop a resistant variety before an epidemic and to utilize the most effective resistance genes in their breeding programmes.

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