Research article

PATHOTYPES DIVERSITY OF *Puccinia striiformis* CAUSING YELLOW RUST OF WHEAT IN NEPAL

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ABSTRACT

Wheat (*Triticum aestivum*) is one of the major stable crop in Nepal. The area of cultivation of wheat is increasing compared to last decade; but its productivity is almost stagnant due to several limiting factors among them, yellow rust caused by *Puccinia striiformis* is one of the major responsible factors. Rust monitoring was carried out to determine the pathotypes diversity of *P. striiformis* during 2018-2021. A total of 109 samples were collected from 41 districts of seven provinces. Each yellow rust samples multiplied in susceptible cultivar and inoculated in differential set for rust pathotypes analysis. The diversity of pathotypes was observed across the country. Seven pathotypes (238S119, 110S119, 46S119, 47S103, 6S0, 78S84 and 110S84) were recorded with different severity level. The pathotypes 46S119, 110S119 and 238S84 found as predominant in different locations. The severity level ranged from resistant to highly susceptible depending on location and genotypes. The severity level was comparatively higher in western-midhill than eastern-mid-hill. The change in pathotypes diversity may be resulted by selection pressure due to the adoption of new wheat genotypes. Monitoring rust pathotypes is essential to develop a strategic plan for yellow rust management by deploying resistant gene/s in wheat genotypes.

***Keywords**: Monitoring, pathotypes, Puccinia striiformis, wheat, yellow rust*

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INTRODUCTION

Wheat (*Triticum aestivum* L.) is third important crop in Nepal after rice and maize. It is cultivated in 0.704 million ha with the total production of 2.01 million mt and the average productivity of 2.85 t/ha (MoAD, 2020). It covers 22% of total cultivated area with 17.3% of the total cereal production, where hilly region covers 44% of the total wheat production. The area of cultivation of wheat is increasing but its productivity is not increasing due to various limiting factors. Among them, diseases are major threat to wheat production in Nepal. Fungal diseases of wheat such as rusts and foliar blight are the primary concerns for wheat production in Nepal. These pathogens are extremely destructive, highly mobile, and pose an imminent threat to millions of vulnerable smallholder farmers. In Nepal, both yellow and leaf rust possess a threat for wheat production in mid/high-hills and Terai regions, respectively. Rust fungi are obligate parasites and must survive on living plants.

In Nepal, the yellow rust disease caused by *Puccinia striiformis* was first recorded in 1964 AD (Karki *et al*., 2004). The first sign of stripe rust is the appearance of yellow streaks (pre-pustules), followed by small, bright yellow, and elongated uredial pustules arranged in conspicuous rows on the leaves, leaf sheaths, glumes and awns. The mature uredial pustules are proliferated and release yellow-orange masses of urediniospores on the leaf surface. In some varieties, long, narrow yellow stripes will develop on leaves. The infected tissues may become brown and dry as the plant matures or becomes stressed. Severe early infection can result in plant stunting (Chen *et al*., 2014).

In mid 1980, the virulence of the rust pathogen began to change and epidemic of rust was encountered due to the prevalence of 7E150 pathotype and RR21 became susceptible to existing pathotype (Sharma *et al*., 1995). Then, continuous considerable research was carried out with an objective to manage newly emerged yellow rust pathotypes, and the research effort developed several high yielding and resistant genotypes in Nepal. As a result, Annapurna 1, Annapurna 3, and Annapurna 4 with Yr9 gene and Annapurna 2, and Kanti with Yr2 gene were released in 1988-1991. Again, in mid-1990, the Yr9 virulent pathotype that evolved in the East African Highland during 1980s and migrated to South Asia from North Africa to Middle East, Central and West Asia causing epidemics on various cultivars of wheat possessing resistance gene Yr9 (Singh *et al*, 2004). The genotype with Yr9 gene such as Annapurna 1, Annapurna 4, and Kanti resistant to the pathotype 7E150 again showed susceptible by yellow rust disease due to appearance of new epidemic *P. striiformis* pathotype (Pst) 46S119 in Baglung, Parbat, Mygdi, and other districts during 1996/97 (Sharma, 2001). Currently, some resistant genotypes released for wheat growing area having Yr27 gene located on chromosome 2BS in the country (Singh *et al*., 2004). Similarly, appearance of another pathotype 71E32, resulted Yr27 containing genotypes and CIMMYT germplasms are becoming ineffective for this pathotype (PPD, 2010). Yellow rust pathotype 46S119 was identified in many samples, followed by 78S84. The pathotyep 46S119 is virulent to Yr9 and pathotype 78S84 is virulent to Yr27 gene (PPD, 2013). However, the Yr9 gene showed resistant to 71E32 on which Yr27 is susceptible (PPD,

2014). The year-round epidemic of new pathotype in the wheat growing areas might be due to spread of fungal spores in the common epidemiologic zone.

MATERIALS AND METHODS

Extensive survey and surveillance was carried out across wheat growing areas in Nepal using Global Positioning System (GPS) to monitor the virulence spectrum of various pathotypes of yellow rust in 2018-21. The survey were done in wheat growing fields including both farmers and trial fields randomly from 41 districts of the seven provinces from the elevation of 69 m to 2943 m above sea level.The surveyed districts were Jhapa, Ilam, Morong, Sunsari, Saptari, Udayapur, Dhanusha, Mohatori, Siraha, Sarlahi, Makawanpur, Chitwan, Nawalparasi, Rupendehi, Pyuthan, Salyan, Dang, Doti, Dailekh, Banke, Bardiya, Jumla, Baglung, Kaski, Parbat, Mygdi, Syanja, Tanahu, Dhading, Kathmandu, Lalitpur, Bhaktapur, Kavre, Dolkha, Sindhupalchok, Mustang, Dhankuta, Nuwakot, Ramechhap, Manang and Terhathum. Rust infected leaves were folded such that rust pustules preserved inside without destroying spores. Excess leaf moisture was removed by keeping them in room temperature with shade drying. Proper dried samples were kept in paper envelope and diagnosed pathotypes at Indian Council of Agricultural Research (ICAR)-Indian Institute of Wheat and Barley Research (IIWBR), Regional Station, Flowerdale, Shimla, India. Uredospores were revived on 2% water agar and multiplied in susceptible host A-9-30-1. The fresh urediospores of each sample were collected from the susceptible host and inoculated on 5-7 days old seedlings of the differential sets in the glass house chamber (Table 1). Based on infection type reaction in differential lines, pathotypes were analyzed from the leaf samples. The infection type matrices of the pathotypes were compared with those of the differential wheat seedling (Nayer *et al*., 2001).

Table 1. Constitution of differential stes 0, A and B for designation of yellow rust pathotypes of wheat rust pathogen (*P. striiformis*)

Set 0	Set A	Set B
WH147	Chinese 166 (*Yr*1)	Hybrid 46 (*Yr*4)
Bilara 2	Lee (*Yr*7)	Heines VII (*Yr*2+)
WH416	Heines Kolben (*Yr*6)	Compare (*Yr*8)
HD2329	Vilmorin 23 (*Yr*3)	*T. spelta album* (*Yr*5)
HD2667	Moro (*Yr*10)	Tc*6/*Lr*26 (*Yr*9)
PBW343	Strubes Dickkopf	Sonalika (*Yr*2+)
HS240	Suwon92 X Omar	Kalyansona *Yr*2 (KS)
Anza	Riebese147/51 (*Yr*9+)	*Yr*24/*Yr*26
A-9-30-1		

RESULTS AND DISCUSSION

Among 109 yellow rust infected samples, 23% was from province 1, 6% was from Medhesh provinde, 19% from Bagmati province, 15% from Gandaki province, 23% from Lumbini province, 6% from Karnali and 8% from Sudurpashchim province. The severity level of yellow rust disease was varied according to location (province) and year. In 2020, yellow rust pathotypes were analysed from different samples for yellow rust. In which, four diferent yellow rust pathotypes 60S0, 238S119, 110S119, 46S119 were detected. Among these pathotypes, 46S119 were dominated in many areas. All these three pathotypes 238S119, 110S119, 46S119 were also detected in Pyuthan district. The diversity of pathotypes of pathogen was observed in region and altitude of the country. In total seven pathotypes (238S119, 110S119, 46S119, 47S103, 6S0, 78S84 and 110S84) were recorded with different severity level from different part of the country. Among them, the pathotypes 46S119, 110S119 and 238S84 were found predominant in many locations as compare to others. The pathotypes 6S0, 238S119, 110S119, 47S103 and 46S119 were commonly recorded from Western parts of the country, whereas pathotypes 6S0, 110S119, 110S84 and 46S119 were dominant at eastern part of the country. Likewise, pathotypes 46S119, 78S84, 110S84 and 78S84 were common at central region. The pathotypes 6S0 was recorded at high altitude area up to 2900 masl. The pathotypes (Pst) 46S119 and 110S119 recorded across the country (Table 2). The pathotypes 47S103 and 6S0 were recorded at few places of central and western parts of the country. Pts 238S119 was reported as newly pathotype in the Nepal which became virulent to most of the genotypes cultivating in the country. The two major Pts 238S119 and 46S119 were found most destructive pathotypes in almost all the commonly growing wheat genotypes such as Vijaya, WK 1204, Dhaulagiri, Sworgadwari, NL 297, Gautam, Bhadganga, NL 971, Danphe, and Achyut.

Three different pathotypes 6S0, 47S103 and 110S119 of *P. striiformis* were recorded of yellow rust pathogen during 2018/19. Similarly, 238S119, 110S119, 46S119, and 110S84 were recorded in 2019/20. In 2020/21, the pathotypes viz. 238S119, 110S119, 46S119 and 6S0 were dominant. The Pts 110S119 was recorded every year at moderate to high frequency level across the wheat growing areas. However, incidence of 46S119 was high in frequency as compare to other pathotypes. The level of incidence of pathotypes was categorized in four different level from low to very high in term of their incidence in different locations/districts i.e., upto two districts as low, four districts as medium, six district as high and more than six districts as very high level of pathotypes distribution in the country (Table 3).

Table 2. List of yellow rust pathotypes analysis at IIWBR, DWR, Shimla, India in 2018-21

S.N.	Location	Genotypes	Score	Pathotypes
1	Rapti Sonora, Dang	Vijaya	60S	6S0
2	ARS, Dailekh	Dhaulagiri	90S	238S119
3	Banguya, Doti	Unknown	60S	238S119
4	Bijuwaphat, Pyuthan	Sawargadwari	60S	110S119
5	Ratamata, Pyuthan	Unknown	60MS-S	238S119
6	Bijuwaphat, Pyuthan	Unknown	60MS-S	46S119
7	Dasarthpur, Surkhet	Banganga	40MS	46S119
8	Kimughau, Dailekh	WK3268	40MS-S	46S119
9	Kavre, Dolkha	Zinsakti Gahun	20S	46S119
10	Jitpurphedio, Kathmandu	WK 2123	40MS	46S119
11	Kusumgaht, Kailali	PBW 343	15MR	238S119
12	Bedkot, Sundarpur	Zinc Gahun	5MS	46S119
13	Gurash, Dailekh	NL 297	30MS	110S119
14	Kavresthali, Kathmandu	NL 297	40MS	46S119
15	Khumaltar, Lalitpur	Swargadwari	20S	46S119
16	Kabre farm, Dolkha	NL 297	40MS	110S84
17	Kushma, Parbat	Unknown	20MS	78S84
18	Godawari, Lalitpur	Unknown	30MS	110S84
19	Melamchi, Sindhupalchok	Gautam	20MS	47S103
20	Sangha, Bhaktapur	Unknown	40M	110S84
21	Jeetpurphedi, Kathmandu	Gautam	20M	110S119
22	Banepa, Kavrepalchok	Unknown	40MS	78S84
23	Panauati, Kavrepalchok	Unknown	60MS	110S119
24	Sipadol, Bhaktapur	Unknown	40MS	46S119
25	Hemja, Kaski	Unknown	20MR	78S84
26	Kaakni, Nuwakot	NL 297	20MS	110S84
27	Sindhuwa, Dhankuta	Unknown	40MS	110S119
28	Kapurkot, Salyan	Gautam	20MR	110S119
29	Manthali, Ramachchap	Unknown	30MS	110S84
30	Jiri, Dolkha	Unknown	20MS	6S0
31	Mude, Sindhupalchok	Unknown	40M	46S119
32	Dhunebesi, Dhading	Unknown	20M	78S84
33	Besi, Mygdi	Unknown	30MS	47S103

Table 3. List of pathotypes of *P. striiformis* recorded with their frequency level in a different part of the country during different years (2018-2020)

S.N.	Pathotype	Year		
		2018-19	2019-20	2020-2021
1	238S119	-	++	+++
2	110S119	+++	+++	++
3	46S119	-	++++	++++
4	47S103	+	-	-
5	6S0	+	-	+
6	78S84	++	-	-
7	110S84	-	++	-

Level of frequency: + = low; ++ = Medium; +++ = High; ++++ = Very high

In 2018, the severity level was medium to high in hill and low at plain area in all the provinces. Similarly, the severity level was also varied in provinces in 2019 and 2020. In province 1, the severity level of yellow rust was low, medium and high with 44%, 29% and 30% respectively during 2019 and the situation was 50% low and 50% high in 2020. Likewise, Madhesh province, disease severity was 14%, 29% and 57% from low, medium and high, respectively in 2019, where the severity were 73%, 14% and 13% low, medium and high, respectively during 2020. In Bagmati province, the severity also varied from low (85%), medium (10%) and high (5%) level and low (73%), medium (14%), and high (13%) during 2019 and 2020 respectively. In Gandaki province, the rust severity was low, medium and high respectively by 57%, 14% and 29% in 2019 which was low (83%) and high by 17% in 2020. In Lumbini province, disease severity was low to high by 83%, 11% and 6% respectively where rust was 73% low, 9% medium and 18% high level in 2019 and 2020 respectively. Likewise in Sudurpaschhim province, the severity of rust situation was 50% at low level and 50% at high level in 2019 and it was 29% low, 57% medium and 14% high level during 2020. Similarly, in Karnali province, the severity was low level in 2019 whereas severity was varied from low to high by 40%, 25% and 35% in 2020 (Fig. 1).

The incidence and severity level were also different with altitudes. Incidence of yellow rust diseases was found more than two third of total samples collected from hill region of the country. Yellow rust recorded in plain area from nearly 15% samples and 13% samples from high hill. In 2019, the incidence of yellow rust was increased in high hill region than during 2020. The 80% yellow rust had moderate to highly susceptible reaction in 2019 and it was reduced by nearly 60% in 2020. In 2020, the severity of yellow rust in plain was low to moderate level whereas the severity was low during 2019. In mid hill, the severity level of yellow rust was medium to high in 2020 while in 2019 the severity level comparably low. Similarly, in high hill, it was moderate level in 2020 but high during 2019. In Nepal, the yellow rust disease usually appear at first in mid and far western regions then it moves

towards easterly. The yellow rust disease was start to appear at first from some places of Kailali then Pyuthan districts. In 2020, it was recorded in popular wheat genotypes Gautam, Bijaya, Chyakhura, Sworgadyari, NL 971, BL 4341, WK 1204 (Table 2).

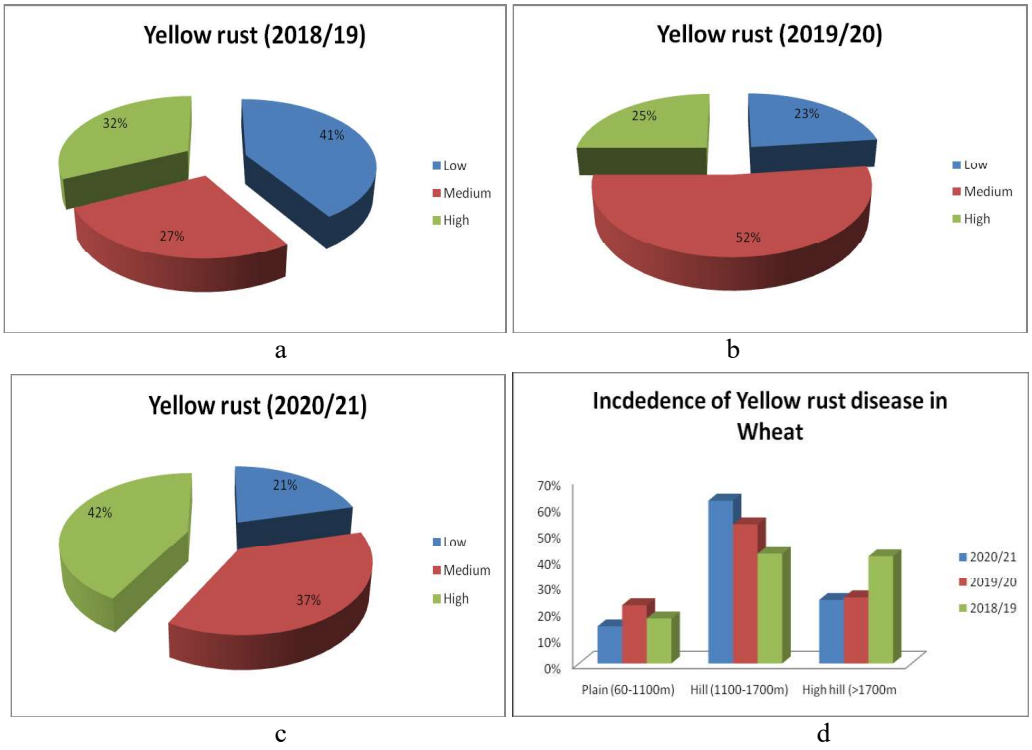

Fig. 1. Incidence and severity of yellow rust disease recorded at different altitudes during different years (a, b, c, d figure from top left to right down as clockwise direction)

Most of the wheat growing season, the prevalence, incidence and severity of diseases and pathotypes was different depending upon the genetic diversity and climate. High diversity of pathogen population structure is observed in the rust pathogens populations in the country. Himalayan region of Nepal could be served as the source for the recurrence of rust for the Indo Gangetic plain and other part of the world (PPD, 2014). Hence, it is need to expand the monitoring and understanding of population in the Himalayan region with collaborative efforts to manage yellow rust at national, regional and global scale. Similarly, monitoring of pathotypes occurring is also necessary for successful planning to manage the rusts by deploying effective genes for resistance. It is also extremely important to keep vigilance of new emerging rust pathotypes virulence and their migration pathway. Side by side, diversification of resistance genes is also needed for strategic planning of the wheat breeding program in future to manage the rust disease in the country and region as a whole.

The frequency of pt.46S119 (virulent to Yr2, Yr3, Yr4, Yr6, Yr7, Yr8, Yr9, Yr17, Yr18, Yr19, Yr21, Yr22, Yr23, Yr25 and YrA) was maximum (42%) followed by pt. 110S119 (29%) and remaining pathotypes were observed in few samples only. Among the seven pathotypes reported on wheat, 46S119 was most frequent and widely distributed followed by 78S84 in mid and far western of Nepal. In previous years, pathotype 78S84 was more prevalent (PPD, 2017). *P. striiformis* population was found avirulent on Yr5, Yr10, Yr15 and YrSp till date. Commonly, yellow rust enters from western part of the country through India and Pakistan regions. In addition, rust pathogens also appear at Bagmati province and Gandaki province could be due to recurrence of pathogen of internal sources from high hill ranges. The frequently of two pathotypes 110S119, and 238S119 were also recorded many parts of India (Anonymous, 2021a; Anonymous, 2021b). The Pst. 238S119, the most virulent and spread pathotype in India, has now might be spread to Nepal through far and mid-western part of the country. Similarly, most of the stripe rust samples of wheat analyzed from Punjab, followed by Himachal Pradesh and Haryana had highest frequency of pathotype 238S119 (49.57%) followed by 110S119 (29.41%) (Anonymous, 2021b). In 2019-20, the prevalence pathotypes of *P. striiformis* were avirulent on *Yr10, Yr25, Yr5, Yr6, Yr7, Yr8, Yr9, Yr15, Yr19, Yr25, Yr33, YrSP, YrSK, YrT* and *YrJ* whereas virulent on *Yr1, Yr2, Yr3, Yr4, Yr7, Yr22, Yr23, Yr17, Yr32, YrA, YrND, YrSU* and *YrCV* gene/s. Whereas, the gene/s *Yr1, Yr6, Yr7, Yr8, Yr17, Yr18, Yr32, Jupateco S, Avocet R(YrA), Avocet S, Yr1, Yr7, Yr8, Yr17, Yr18, Yr24, Yr32, YrJupateco S and YrA, Yr5, Yr9, Yr10, Yr15, Yr24, Yr26, Yr27, YrSp, YrJupateco R* were virulent with pathotypes (PPD, 2021). Similarly, gene/s *Yr5+, Yr6+, Yr9+, Yr10+, Yr15+, Yr26, Yr27, YrJupateco R* and *YrSp+* were became avirulent reaction (PPD, 2020). Likewise, in 2018/19, *Yr5*, *Yr9*, *Yr10*, *Yr15*, *Yr24*, *Yr26*, *Yr27*, *YrSp* and *Yr18* (JupatecoR) were found effective to yellow rust disease against the pathotypes, while gene(s) *Yr1*, *Yr6*, *Yr7*, *Yr8*, *Yr17*, *Yr18*, *Yr32* and *YrA* were ineffective. Similarly, the pathotypes 46S119, 110S119 were found as *Yr9* and *Yr27* virulence respectively (PPD, 2020).

Newly released wheat genotypes WK 1712 (Mudule 1), WK 2286 (Suruma), WK 2370 (Tela), WK 2422 (Kautila), WK 3027 (Khumal Shakti), WK 3026 (Himganga), WK 2748 (Bheriganga), Borlaug 2020, NL 1327 (Zinc Gahun 1), NL 1369 (Zinc Gahun 2) were found tolerant against the pathotypes 46S119, 238S119, 110S119 and 238S84. Where, older genotypes, Vijaya, WK 1204, Dhaulagiri, Banganga, WK2123, Gautam, Danphe, NL 297, NL 971 were susceptible due to evolution of diversity of pathogens (PPD, 2020). Similarly, Khan *et al.*, (2019) study showed that the genetic structure of *P. striiformis* showed from Nepal (35 isolates) and Bhutan (31 isolates) in comparison with 81 Pakistani samples collected during 2015 and 2016, through microsatellite genotyping. They revealed a recombinant and highly diverse population structure in Pakistan, Bhutan and Nepal. A high level of genotypic diversity (>0.90) was observed for all the three countries Pakistan (0.96), Bhutan (0.96) and Nepal (0.91) with the detection of 108 distinct multilocus genotypes (MLGs) in the overall population; 59 for Pakistan, 27 for Bhutan and 26 for Nepal. Hence, Nepal could also be the potential sources of rust epidemic for whole Indian sub-continent

and also other parts of the world. These reports indicate the importance of continuous survey and survuilane for rust diseases in Nepal.

For early detection of wheat rust, it needs to monitor from end of January at western part of the country. Timely monitoring is important to set strategies to manage disease. Presence of yellow rust in plain area shows the potential emergence of heat tolerant *P. striiformis* pathotype/s in Terai region of Nepal. In addition, circulation of message based on continuous survey and surveillance, advisory massage alert through mobile massage, advisory bulletin and others e-news to the concern stakeholders specially including farmers and fields technicians had also taken as next steps for the management of disease on time prior to distribute many places of the country by applying systemic fungicides. Continuous testing of exotic genotypes having resistant gene/s and monitoring of new pathotypes are important tool to combat against yellow rust disease in whole south Asian region. Earlier genotype selection was based on major gene however recent approach is needed for selection of slow rusting genotype/s for long term durability combination with minor gene/s. So, monitoring of rust diseases should be done continuously and systematically in future to cope with the threat to wheat production in the country.

CONCLUSIONS

Yellow rust widely occurs across the country and the scenario of rust pathotypes and their reaction are usually varied in every year depending upon the genetic diversity of wheat genotypes cultivated and environment. Surveillance is one of the important task to determine the status of wheat diseases in the country. As the yellow rust pathogen is obligate nature so its mysterious reoccurrence in every year during the off-season with surviving either on alternate host or collateral hosts in different location of the country is still need to confirm in the country. Monitoring the pathotypes occurring is a necessary step for successful planning to manage the rusts by deploying effective genes for resistance or by using other means of control on time. It is also extremely important to keep vigilance of new emerging rust pathotypes virulence in the country. This in turn could increase national production and productivity of wheat.

ACKNOWLEDGEMENT

The authors thankful to Nepal Agricultural Research Council (NARC) for financial & administrative support to execute the research work. We also special thank to Dr. S.C. Bhardwaj, Principal Scientist and his team, Indian Institute of Wheat and Barley Research (IIWBR), Indian Council of Agricultural Research (ICAR), Regional Station, Flowerdale, Shimla, India for analysis of pathotypes of wheat samples. We also express sincere acknowledge to Dr. David Hodson, CIMMYT- Maxico for providing financial grant and technical guidance for monitoring disease and sample collection.

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