JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Dec. 2017. Vol. **11**(4), p. 1839-1844

Germplasm Evaluation and Characterization of Slow Rusting Resistant Gene against Stripe Rust of Wheat

Saima Farooq, V.K. Razdan, Vishal Gupta*, Kausar Fatima, Seethiya Mahajan and Satish Sharma

Division of Plant Pathology, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Chatha, India.

http://dx.doi.org/10.22207/JPAM.11.4.23

(Received: 16 October 2017; accepted: 09 December 2017)

Thirty one wheat germplasm lines were screened under natural epiphytotic conditions against stripe rust at University Research Farm, Chatha, during *Rabi*, 2014-15. On the basis of final rust severity (FRS), area under rust progress curve (AURPC) and coefficient of infection (CI), eight lines (Raj 4037, Sonara 64, NP 823, HPW 42, K9351, NIAW 301, PBW 12, and HUW 213) exhibited partial resistance to the disease while as on the basis of infection rate (r) six lines (NP 825, HP 1633, K8434, PBW 12, Ajanta and K9533) showed partial resistance to the disease. Field response of these lines against stripe rust showed that 14 genotypes (Sonara 64, Utkalia, NI 5439, NIAW 301, PBW 12, HUW 213, Ajanta, NP 823, K8434, K9533, Sharbati Sonara, Raj 4037, HP 1633, HPW 42 and K9351) were moderately resistant, 16 (Tawa, KRL, RW 346, HD 2643, HS 1097, NP 825, WH 291, HUW 12, PBW 226, NI 179, NI 5439, K9644, HD1925, PBW 65, PV 18 and GW 503) were moderately susceptible and one genotype (Agra Local) was susceptible. Assessment of losses was also calculated at different growth stages and it was observed that losses at flowering stage and dough stage were highest in one line (Agra Local). Nine wheat germplasm lines (Sonara 64, K9351, HP 1633, Raj 4037, Sharbati Sonara, K9533, K8434, NP 823 and Ajanta) amplifying a band of 523 bp fragment indicating presence of *Yr18* gene.

Keywords: Stripe rust, Severity, Allele specific marker, *Yr18*, FRS, AURPC, CI, r, *Cssfr2*.

India is a privileged country to attain and retain the status of being the second largest producer of wheat and registering the historic production of 93.50 million tonnes during 2015-16. Among the three rust, stripe or yellow rust caused by *Puccinia striiformis* Westend f. sp. *tritici* is devastating foliar disease and is considered of immense importance in successful cultivation of wheat (Singh *et al*., 2014). Year after year the susceptible wheat cultivars that suffer from stripe rust disease result in increased inoculum build up thus posing major threat to wheat cultivation. Although remarkable progress has been made in breeding for stripe rust resistant varieties but the subsequent evolution of pathogen races at much greater pace continues to challenge this breeding programme (Singh *et al*., 2011) and stripe rust remains a threat to wheat cultivation (Sareen *et al.,* 2012). Although the timely application of fungicides against this obligate parasite can manage the disease to some extent but their use add to the production costs. Breeding for resistance remains the most effective and efficient management strategy as it does not add input costs to farmers and is environmentally safe (Yang and Liu, 2004). The identification and knowledge of the resistance genes in commonly used parental germplasm and released cultivars is very important for utilizing the genetic resistance to manage this rust in full potential. The long term and economical

* To whom all correspondence should be addressed.
E-mail: vishalguptapgpr@gmail.com

strategy could thus be resistance breeding through deployment of effective rust resistance genes over space and time (Pretorius *et al.*, 1997; Zeng *et al.,* 2014). The genes expressing at adult plant stage have special significance because the cultivars having such genes have shown partial resistance that has remained effective for longer durations (Singh and Rajaram, 1991; Khan and Saini, 2009).

The outbreak of stripe rust in temperate areas of Jammu and Kashmir State, India is a matter of great concern owing to the fact that the rust inoculum generated in these areas may act as a reservoir of inoculum for rust initiation in North-West Plain Zones of the country. Therefore there is urgent need to curtail the pathway of rust pathogen in Jammu and Kashmir State on priority basis so that the same may not spread to food bowl of the country thereby causing a great threat to the National Food Security Mission (NFSM). With this background information the present study was undertaken to screen the wheat germplasm for identifying stripe rust resistant genotypes and then validate resistance gene (*Yr18)* at molecular level.

MATERIALS AND METHODS

The present studies were carried out in the Division of Plant Pathology, Faculty of Agriculture, SKUAST Jammu, Chatha, during *rabi* 2014-2015.

Screening of wheat germplasm for slow rusting traits

Thirty one wheat genotypes *viz*., Sonara 64, Utkalia, NI 5439, NIAW 301, PBW 12, HUW 213, Ajanta, NP 823, K8434, K9533, Sharbati Sonara, RAJ 4037, HP 1633, HPW 42 , K9351, Tawa, KRL, RW 346, HD 2643, HS 1097, NP 825, WH 291, HUW 12, PBW 226, NI 179, K9644, HD1925, PBW 65, PV 18, GW 5031 and Agra Local) were used for screening for stripe rust disease under natural epiphytotic conditions during *rabi* 2014-2015 at University Research Farm, Chatha (32^{o} 43' N, 74^{o} 54' E). Each collected genotype was grown in three rows 2m apart with spacing of 22.5cm in third week of November, 2014. The entire experimental field was surrounded by the susceptible genotype Agra Local. The observations regarding per cent stripe rust severity were recorded from 29th Jan. 2015, onward till the crop was harvested. The modified Cobb's scale which gives combined scores for level of severity and infection type was adopted to record the disease severity. Observations for slow rusting traits such as Final rust severity (FRS), Area Under Rust Progress Curve (Milus and Line, 1986), Coefficient of infection and Infection Rate (Brorers, 1989) were recorded.

Molecular validation of stripe rust resistance gene in wheat germplasm

Nineteen wheat genotypes (Sonara 64, K 9351, HP 1633, Raj 4037, Sharbati Sonara, K 9533, K 8434, NP 823, Ajanta, PBW 12, KRL, RW 346, HD 2643, HS 1097, NP 825, PBW 226, NIAW 301, PBW 343, and NI 179) were selected on the basis of low disease severity under field conditions for molecular validation of stripe rust resistance gene *Yr18* by using allele-specific markers *Cssfr2* (F=TTGATGAAACCAGTTTTTTTTCTA R=TATGCCATTTAACATAATCATGAA). The genomic DNA of selected genotypes was isolated by CTAB (cetyl-trimethyl ammonium bromide) method (Doyle and Doyle, 1987). Polymerase chain reaction (PCR) ampliûcation was conducted by *Cssfr2* primer with temperature proûles as described by Laugdah *et al.* (2009). The ampliûcation products were separated on 3 per cent agarose gels containing ethidium bromide and 1× TBE buûer. The gels were visualized using gel documentation system for documentation of allele type in selected genotypes for resistance gene *Yr18* using a standard molecular ladder of 100 bp.

Assessment of losses caused by stripe rust

Assessment of per cent losses due to the stripe rust in case of thirty one test genotypes *viz.*, Tawa, KRL, Raj 4037, RW 346, SONARA 64, Utkalia, HD 2643, HS 1097, NP 825, WH 291, HP 1633, HUW 12, PBW 226, NP 823, PV 18, K 8434, NI 179, HPW 42, NI 5439, K9644, K9351, HD1925, NIAW 301, PBW 12, PBW 65, HUW 213, Ajanta, K9533, GW 503, Sharbati Sonara and Agra Local, were estimated by working out the per cent losses at different phonological stages of wheat crop, such as tillering, flowering and milking Mundy's equation (1973) as : Loss = (0.44 x disease severity) + 3.15

RESULTS AND DISCUSSION

Screening of wheat germplasm for slow rusting traits

During the present studies thirty-one

genotypes of wheat were screened for their resistance and susceptibility response against stripe rust disease under subtropical agroclimatic conditions of Jammu. The disease was first observed on 4th Feb., on a susceptible host Agra Local. Subsequently, the disease was recorded in other tested genotypes as well, but it was on 25th Feb. that the disease engulfed all the test genotypes and the disease severity ranged from 5-25 per cent. Thereafter the disease severity increased at a steady pace till 25th Mar. which was considered as the Final Rust Severity (FRS). The FRS ranged from 25 to 95 per cent, with maximum disease severity (95%) in Agra Local and minimum of 25 per cent each in K9351, NIAW301, PBW 12 and HUW213 (Table 1). Bariana *et al.* (2002) screened 176 doubled haploid lines derived from CD87/Katepwa (CD/K) for stripe rust resistance. Both parental lines, CD87 and Katepwa, showed stripe rust resistance. Both lines were susceptible to 110 E143A+ at seedling stage. The presence of susceptible segregates indicated the genetic independence of resistance in CD87 and Katepwa. Data in Table 1 further exhibited that AURPC value in 9 genotypes (Raj 4037, Sonara 64, HS 1097, NP 823, HPW 42, K 9351, NIAW 301, PBW 12 and HUW 213) ranged from 0-800 and in 21 genotypes (Tawa, KRL, RW 346, Utkalia, HD 2643, NP 825,

Table 1. Evaluation of wheat germplasm for slow rusting parameters against *Puccinia striiformis*

S. No.	Germplasm	Final Rust Severity (FRS) (AURPC)	Area Under Rust Progress Curve	Coefficient of Infection (CI)	Infection rate (r)
01	Tawa	40	1040	30.00	0.036
02	KRL	40	960	30.00	0.025
03	RAJ 4037	30	604	07.50	0.084
04	RW 346	40	882	30.00	0.036
05	Sonara 64	30	606	07.50	0.049
06	Utkalia	40	842	30.00	0.036
07	HD 2643	45	984	33.75	0.039
08	HS 1097	40	644	30.00	0.056
09	NP 825	45	1300	33.75	0.014
10	WH 291	45	1020	33.75	0.039
11	HP 1633	30	1020	07.50	0.017
12	HUW 12	40	922	30.00	0.036
13	PBW 226	40	962	30.00	0.036
14	NP 823	30	726	07.50	0.028
15	PV 18	40	926	30.00	0.036
16	K8434	30	924	07.50	0.017
17	NI 179	45	864	33.75	0.039
18	HPW 42	30	644	07.50	0.049
19	NI 5439	45	902	33.75	0.039
20	K9644	40	886	30.00	0.036
21	K9351	25	626	06.25	0.044
22	HD 1925	45	984	33.75	0.028
23	NIAW 301	25	648	06.25	0.024
24	PBW 12	25	758	06.25	0.019
25	PBW 65	50	1362	37.50	0.023
26	HUW 213	25	666	06.25	0.024
27	Ajanta	30	978	07.50	0.017
28	K9533	30	820	07.50	0.017
29	GW 503	45	1022	33.75	0.028
30	Sharbati sonara	35	1046	08.25	0.021
31	Agra Local	95	2340	95.00	0.122

Table 2. Response of wheat germplasm against stripe rust under field conditions

S. No.	Disease response	Genotype
1	Immune	Nil
2	Nearly immune	Nil
3	Resistant (R)	Nil
4	Moderately resistant (MR)	SONARA 64 Utkalia NI 5439 NIAW 301 PBW 12 HUW 213 Ajanta NP 823 K8434 K9533 Sharbati Sonara Raj 4037 HP 1633 HPW 42 and K9351
5	Moderately susceptible (S)	Tawa KRL RW 346 HD 2643 HS 1097 NP 825 WH 291 HUW 12 PBW 226 NI 179 NI 5439 K9644 HD1925 PBW 65 PV 18 and GW 503
6	Susceptible	Agra Local

WH 291, HP 1633, HUW 12, PBW 226, PV 18, K 8434, NI 179, NI 5439, K 9644, HD 1925, PBW 65, Ajanta, K 9533, GW 503 and Sharbati Sonara) from >800 to 1600, however, in Agra Local it was highest (2340). While calculating the CI, a value of 6.25 to 8.25 was observed in Sonara 64, NIAW 301, PBW 12, HUW 213, Ajanta, NP 823, K 8434, K 9533, Sharbati Sonara, HP 1633, HPW 42, K 9351 and Raj 4037 and 30 to 33.75 in Tawa, KRL, RW 346, Utkalia, HD2643, HS 1097, NP 825, WH 291, PBW 226, PV 18, NI 179, NI 5439, K9644, HD 1925, PBW 65 and GW 503 , whereas,

Table 3. Molecular validation of *Yr18* for yellow rust

S. No.	List of Wheat Germplasm lines	*Yr18* (+/-)
1.	Sonara 64	+
2.	K9351	+
3.	HP 1633	+
4.	Raj 4037	+
5.	Sharbati Sonara	+
6.	K9533	+
7.	K8434	+
8.	NP 823	+
9.	AJANTA	+
10.	PBW 12	-
11.	KRL	-
12.	RW 346	-
13.	HD 2643	-
14.	HS 1097	-
15.	NP 825	-
16.	PBW 226	-
17.	NIAW 301	-
18.	PBW 343	-
19.	NI 179	-

Table 4. Assessment of losses in wheat germplasm due to stripe rust (*Puccinia striiformis*) of at different growth stages

S.. No	Genotype	Per cent losses		
		Tillering stage	Flowering stage	Dough stage
01	Tawa	0	16.35	20.75
02	KRL	0	16.35	20.75
03	RAJ 4037	0	11.95	16.35
04	RW 346	0	16.35	20.75
05	Sonara 64	0	14.15	16.35
06	Utkalia	0	16.35	20.75
07	HD 2643	0	20.75	22.95
08	HS 1097	0	14.15	20.75
09	NP 825	0	20.75	22.95
10	WH 291	0	16.35	22.95
11	HP 1633	0	16.35	16.35
12	HUW 12	0	16.35	20.75
13	PBW 226	0	16.35	20.75
14	NP 823	0	14.15	16.35
15	PV 18	0	16.35	20.75
16	K8434	0	16.35	16.35
17	NI 179	0	16.35	22.95
18	HPW42	0	14.15	16.35
19	NI 5439	0	16.35	22.95
20	K9644	0	16.35	20.75
21	K9351	0	14.15	14.15
22	HD1925	0	16.35	22.95
23	NIAW 301	0	11.95	14.15
24	PBW 12	0	11.95	14.15
25	PBW 65	0	22.95	25.15
26	HUW 213	0	11.95	14.15
27	Ajanta	0	14.15	16.35
28	K9533	0	16.35	16.35
29	GW 503	0	16.35	22.95
30	Sharbati Sonara	0	16.79	18.55
31	Agra Local	0	36.15	44.95

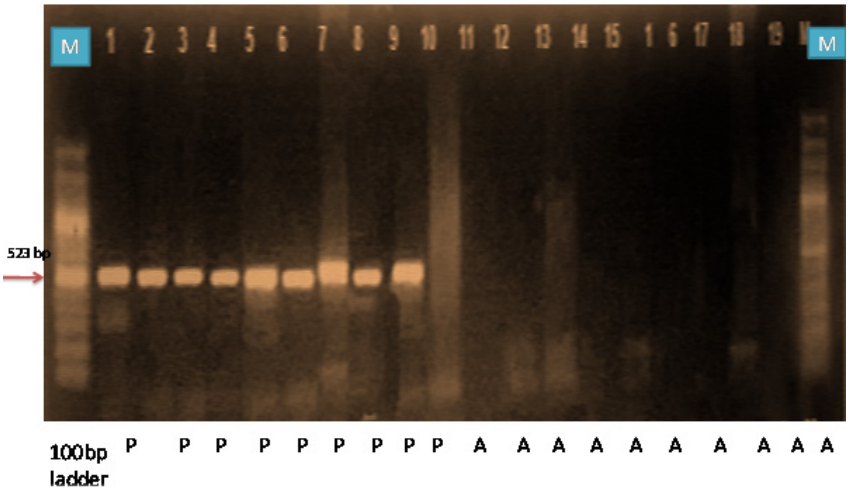
in Agra Local the CI value (95) was the highest. Similarly, the infection rate (r) varied from 0.014 in case of NP 825 to 0.122 in Agra Local. Among them six germplasm (NP 825, HP 1633, K8434, PBW 12, Ajanta and K9533) exhibited a range of 0.014-0.019, whereas, in eight genotypes (KRL, NP 823, HD 1925, NIAW 301, PBW 65, HUW 213, GW 503, and Sharbati Sonara) the infection rate ranged from 0.021 to 0.028. In case of TAWA, RW 346, Sonara 64, Utkalia, HD 2643, WH 291, HUW 12, PBW 226, PV 18, NI 179, HPW 42, NI 5439, K9644, and K9351 the infection rate ranged from 0.036 to 0.049. However, the infection rate in case of HS 1097, Raj 4037 and Agra Local was comparatively high (0.056, 0.084 and 0.122, respectively). The result was in conformity with the findings of other workers who reported that breeding lines with low value of AURPC, CI and r were expected to possessed genes that conferred partial resistance (Ali *et al.,* 2012). Data in Table 2 indicate that genotypes were categorized as moderately resistant (Sonara 64, Utkalia, NI 5439, NIAW 301, PBW 12, HUW 213, Ajanta, NP 823, K 8434, K 9533, Sharbati Sonara, Raj 4037, HP 1633, HPW 42 and K9351), moderately susceptible (Tawa, KRL, RW 346, HD 2643, HS 1097, NP 825, , WH 291, HUW 12, PBW 226, NI 179, NI 5439, K9644, HD1925, PBW 65, PV 18 and GW 503) and susceptible (Agra Local), whereas, none of the tested genotypes showed resistant response against stripe rust

Molecular validation of stripe rust resistance gene in wheat germplasm

The *Yr18* locus confers partial and durable adult plant resistance against stripe rust fungus. In order to track *Yr18*, PCR was carried out with an allele specific primer *cssfr2* which resulted in the amplification of 523 bp fragment, which exhibited the presence and absence of banding pattern. Nine wheat genotypes (Sonara 64, K 9351, HP 1633, Raj 4037, Sharbati Sonara, K 9533, K 8434, NP 823 and Ajanta) amplified a band of 523 bp fragments which indicated the presence of *Yr18* gene (Table 3, Fig., 1). Five allele-specific markers (*cssfr1–cssfr5*) were developed by Lagudah (2009) based on a 3 bp deletion in exon 11 of the *Yr18*-gene which were closely linked to the *Lr34*/*Yr18*/*Ltn1*/*Pm38* locus and have been shown to provide a much wider diagnostic ability for this multi-pathogen resistance trait in diverse wheat cultivars. The validation of results are in accordance with the findings of Laugdah *et al.* (2009).

Assessment of losses caused by stripe rust

While assessing the losses while evaluating thirty one genotypes at tillering, flowering and dough phonological stages, though there was no loss at the initial stage of tillering, but the per cent losses at flowering stage ranged from 11.95 to



M = Ladder Samples - 1 (SONARA 64) 2 (K9351) 3(HP 1633) 4(Raj 4037) 5(Sharbati Sonara) 6(K9533) 7(K8434) 8(NP 823) 9 (Ajanta) 10(PBW 12) 11(KRL) 12 (RW 346) 13(HD 2643) 14(HS 1097) 15(NP 825) 16(PBW 226) 17(NIAW 301) 18(PBW 343) 19(NI 179)

Fig. 1. PCR amplification of wheat germplasm by cssfr2

36.15. In case of Raj 4037, NIAW 301, PBW 12 and HUW 213 the per cent losses estimated were 11.95, whereas, the per cent losses estimated were 20.75 (HD 2643 and NP 825), 22.95 (PBW 65) and 36.15 (Agra Local). Similarly, at the dough stage the per cent losses estimated in K 9351, NIAW 301, PBW 12 and HUW 213 was 14.15, whereas, in case of PBW 65 and Agra Local the losses recorded were 25.15 and 44.95 per cent, respectively. Chen (2005) have reported that the disease may cause 40-78 per cent losses under normal conditions, but if the conditions were favorable for disease spread the loss might rise to 84 per cent (Murray *et al.,* 1994). The predicted losses may be less if the wheat varieties were resistant or slow rusting against rust diseases (Salman *et al.,* 2006). Similarly, the wheat varieties which showed less area under disease progress curve normally bear less yield losses (Hussain *et al.,* 1996).

REFERENCES

1. Ali, S., Leconte, M., Delos, M., Enjalbert, J and de, Vallavieille-Pope. Virulence dynamics and regional structuring of *Puccinia striiformis* f. sp. *tritici* in France between 1984 and 2009. *Pl. Dis.*, 2012; **96**: 131-140.
2. Bariana, H. S., Brown, G. N., Ahmed, N. U., Khatkar, S., Conner, R. L., Wellings, C. R., Haley, S., Sharp, P. J and Laroche, A. Characterization of *Triticum vavilovii* derived *stripe* rust resistance using genetic, cytogenetic and molecular analyses and its marker-assisted selection. *Theo. App. Gen.,* 2002; **104**: 315-320.
3. Broers, L. H. M. Partial resistance to wheat leaf rust in 18 spring wheat cultivars. *Euphy.*, 1989; **44**: 247-258.
4. Chen, X. M. Epidemiology and control of stripe rust (*Puccinia striiformis* f. sp. *tritici)* on wheat. *Can. J. Pl. Path.,* 2005; **27**: 3.
5. Doyle, J. J and Doyle, J. L. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.,* 1987; **19:** 11-15.
6. Hussain, M., Mumtaz, H and Shah, J. A. Wheat rust scenario in 1994-1995. *J. Phytopatho.,* 1996; 96-100.
7. Khan, M. A and Saini, R. G. Non-hypersensitive leaf rust resistance of bread wheat cultivar PBW 65 conditioned by genes different than *Lr34*. *Czech J. Gen.Pl Breed.,* 2009; **45**: 26-30.
8. Lagudah, E. S., Krattinger, S. G., Herrera-Foessel, S., Singh, R. P., Huerta-Espino, J., Spielmeyer, W., Brown-Guedira, G., Selter, L. L and Keller, B. Gene-specific markers for the wheat gene *Lr34/Yr18/Pm38* which confers resistance to multiple fungal pathogens. *Theo. App. Gen.,* 2009*;* **119**: 889-898.
9. Milus, E. A and Line, R. F. Gene action for inheritance of durable, high temperature, adult plant resistance to *stripe* rust in wheat. *Phytopatho.,* 1986; **76**: 435-441.
10. Munday, E. J. The effect of yellow rust and its control on the yield of Joss Cambier Winter Wheat. *Pl. Pathol.,* 1973; **22**: 171-176.
11. Murray, G. M., Ellison, P. J., Watson, A and Cullis, B. R.. The relationship between wheat yield and *stripe* rust as affected by length of epidemic and temperature at grain development stage of crop growth. *Pl. Patho.,* 1994; **43**: 397-405.
12. Pretorius, Z. A., Boshoff, W.H.P and Kema, G.H.J. First report of *Puccinia striiformis* f. sp. *tritici* on wheat in South Africa. *Pl. Dis.,* 1997; **81**: 424.
13. Salman, A., Khan, M. A and Hussain, M. Prediction of yield losses in wheat varieties/ lines due to leaf rust in Faisalabad. *Pakistan J. Phytopatho.,* 2006; **18**: 178-182.
14. Sareen, P., Sundeep, K., Uttam, K., Lakshman, P,, Amit, K.S., Rakesh, S and Joshi, A.K.. Pathological and molecular characterizations of slow leaf rusting in fifteen wheat (*Triticum aestivum L*.) genotypes. *African J. Biotech.,* 2012; **11**: 14956-14966.
15. Singh, R. P and Rajaram, S. Resistance to *Puccinia recondita* f.sp. *tritici* in 50 Mexican bread wheat cultivars. *Crop Sci.,* 1991; **31**: 1472-1479.
16. Singh, R., Huerta-Espino, J., Bhavani, S., Herrera-Foessel, S., Singh, D., Singh, P., Velu, G., Mason, R., Jin, J., Njau, P and Crossa, J.. Race non-specific resistance to rust diseases in CIMMYT spring wheat. *Euphy.* , 2011; **179**: 175-186.
17. Singh, D.P., Sharma, A. K., Babu, K. S and Sharma, I. Multiple diseases, insect pests resistant genotypes and their utilization in breeding for resistance in wheat and *Triticale*. *Greener J. Ag*. Sc. 2014; 150-165.
18. Yang, W. X and Liu, D. Q. Advances in localization and molecular markers of wheat leaf rust resistance genes. *Sci. Ag. Sinica.,* 2004; **3**: 770-779.
19. Zeng, Q.D., Han, D. J., Wang, Q. L and Kang, Z. S. *Stripe* rust resistance and genes in Chinese wheat cultivars and breeding line. *Euphy*., 2014; **196**: 271-284.