RESEARCH ARTICLE

Assessment of wheat varieties for slow rusting and management of stripe rust caused by *Puccinia striiformis*

SEETHIYA MAHAJAN¹, VISHAL GUPTA^{1*}, V.K. RAZDAN¹, KAUSAR FATIMA¹ and SATISH SHARMA²

¹Division of Plant Pathology, Faculty of Agriculture, ²Seed Multiplication Farm, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Chatha 180 009, Jammu & Kashmir, India

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ABSTRACT: Fifty wheat varieties were screened under artificial epiphytotic conditions against stripe rust of wheat during Rabi, 2013-14. On the basis of final rust severity (FRS), AURPC (Area Under Rust Progress Curve), *r* (Infection Rate) and CI (Coefficient of Infection), 10 lines exhibited partial resistance against the disease. PCR profiles of 50 wheat varieties by the allele-specific primer (cssfr1) revealed distinct fragment of 517 bp in 23 varieties indicating the presence of *Yr18* gene. Wheat varieties, PBW 343, DPW 621-50, RSP 561 and HD 2967 were used for testing five fungicides viz., Amistar Top, Quadrix, Score, Folicur and Tilt, each at 0.1%. Amistar Top was found to be most effective in reducing the disease severity of stripe rust in PBW 343, followed by Quadrix, Score, Folicur and Tilt. The application of these fungicides also showed a significant increase in the grain yield, with maximum increase (33.58%) by Amistar Top in HD 2967 followed by Quadrix, Score, Folicur and Tilt.

Keywords: cssfr1, fungicides, *Puccinia striiformis*, stripe rust, wheat

There were recurrent epidemics of stripe rust of wheat in northern western plain zone of India and occurrence of new virulent pathotypes coupled with the susceptibility of cultivars responsible for a significant reduction in grain yield of wheat (Prashar *et al.*, 2007). The disease affects wheat plant through damaging its respiratory system, reduction in photosynthetic area, stunted growth and most importantly reduced grain yield and test weight by shrivelling of grain (Chen, 2005). Losses in the yield of wheat due to stripe rust primarily depend on the level of susceptibility, environmental conditions and the stage of infection (Singh *et al.*, 2002).

Cultivation of resistant varieties is one of the most feasible methods to manage stripe rust which has no human health and environmental hazardous concerns (Chen, 2007; Farrokhi *et al.*, 2011). Although deployment of race-specific resistance genes ensures effective protection against rust diseases (Shah *et al.*, 2010) but they remain effective only for a few years (Lagudah *et al.*, 2009), whereas, race-non-specific resistance or slow rusting or partial resistance appeared polygenic and durable (Herrere-Fossel *et al.*, 2007). The *Lr34/Yr18* gene which confers race-non specific, partial and slow rusting to rusts has appeared effective for the breeding of resistant varieties. Marker *csLV34* closely linked to the *Lr34/Yr18* locus have been shown to be specific diagnostic tools for this multi-pathogen resistance trait (Lagudah *et al.*, 2006). But on the appearance of the disease in the field, application of synthetic fungicides

are widely advocated (Gupta *et al.*, 2013; Selvakumar *et al.*, 2014). Keeping in view these facts, experiments were conducted to evaluate the varieties for slow rusting resistance (genotypic and phenotypic) and also to study the effect of different fungicides in the management of stripe rust in different varieties.

MATERIALS AND METHODS

The investigations on the characterization of wheat varieties for slow rusting resistance against *Puccinia striiformis* and management with fungicides were carried out during *rabi* season of 2013-2015 in the Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Chatha. Materials and methods used for conducting experiments are described as:

Screening of wheat varieties

Fifty wheat varieties (SONORA 64, DWR 16, PBW 343, HD 2281, DURGAPUR 65, VL 616, DBW 16, WH 542, WH 711, GW 496, VL 421, K65, UP 2121, CPAN 3004, RSP 561, HD 2687, HD 2307, SARBATI SONORA, PBW 175, HD 2404, HD 2135, DBW 621-50, HS 240, PBW 373, JAUW 584, HW 2045, RAJ 1482, WH 533, RAJ 821, UP1109, PBW 550, UP 2425, VL 804, LAL BAHADUR, DBW 17, HW 2004, VL 404, HD 2824, PBW 443, WH 1105, SONALIKA, PBW 154, AGRA LOCAL, NP 715, HD 2967, RAJ 3077, RAJ 3765, KALYAN SONA, HD 2329 and HD 2864) were screened against stripe rust under artificial epiphytotic conditions at University

*Corresponding author: vishal94gupta@rediffmail.com

Research Farm, Chatha. The varieties were sown in rows (2.0 m) with spacing of 22.5 cm apart in the first week of November 2013. The whole of the experimental area was surrounded by the susceptible wheat variety (PBW-343). Inoculation of wheat varieties was carried out after 30 days of sowing by urediospore suspension having mixed pathotypes of *P. striiformis* collected from Regional Rust Research Centre (ICAR), Flowerdale, Shimla (H.P.). Data on Final rust severity (FRS), Coefficient of infection (CI), apparent infection rate (r) and AURPC (Area Under Rust Progress Curve) was recorded (Pathan and Park, 2006)

Molecular characterization of Yr18 locus

Molecular characterization of 50 wheat varieties for Yr18 locus was carried out by using allele-specific markers viz., *cssfr1* (F=TTGATGAAACCAGTTTTTTTCTA; R=GCCATTTAACATAATCATGATGGA). DNA extractions, purification and quantification were carried out at the two-leaf stage from each variety. Polymerase chain reaction (PCR) amplification by using the above primer was conducted with temperature profiles as described by Seah *et al.* (1998). The amplification products were separated on 3% agarose gels containing ethidium bromide and 1 × TBE buffer. The gels were visualized using gel documentation system for documentation of allele type in selected variety.

Management by synthetic fungicides

Four wheat varieties (PBW 343, DPW 621-50, RSP 561 and HD 296) were selected based upon their susceptibility response against the stripe rust, sown in experimental plot having 3 m² size consisting of 15 rows, each 2.0 m long and 22.5 cm apart during first fortnight of November, 2013-2015. The experiment was laid out in factorial randomized block design with four replications. Five different fungicides viz., Amistar Top (Azoxystrobin-18.2% + Difenconazole SC), Quadrix (Azoxystrobin-22.9% SC), Score (Difenconazole 25 EC), Folicur (Tebuconazole 250 EC) and Tilt (Propiconazole 25 EC) @ 0.1 per cent concentration were tested for their effectiveness in managing stripe rust in different varieties. A control plot of each variety (with water only) was kept for each treatment. A single spray of respective fungicides was done in the first week of February after the appearance of stripe rust symptoms. Observations regarding disease severity were recorded (at 10 days interval) on the basis of modified Cobb's scale (Peterson *et al.*, 1948) and yield/plot in each treatment was recorded after harvest. The data obtained was statistically analyzed and critical difference (CD) at level p=0.05 was worked out.

RESULTS AND DISCUSSION

Screening of wheat varieties for slow rusting resistance against *P. striiformis*

The data presented in Table 1 revealed that based on FRS (Final Rust Severity), the varieties were categorized into 3 group having low level (1-35%), moderate (36-

Table 1. Screening of wheat varieties against stripe rust of wheat

Varieties	*FRS	**AURPC	**r	***CI
AGRA LOCAL	90	2325	0.135	70.00
CPAN 3004	40	1425	0.101	32.00
DBW 16	40	850	0.098	32.00
DBW 17	50	1675	0.129	50.00
DPW 621-50	40	1195	0.069	42.00
DWR 16	60	1825	0.149	60.00
DURGAPUR 65	40	1675	0.129	50.00
GW 496	42	1775	0.136	60.00
HD 2329	30	750	0.071	25.00
HS 240	40	900	0.120	32.00
HD 2307	25	750	0.132	10.00
HD 2824	50	1000	0.114	50.00
HD 2864	30	650	0.081	39.00
HD 2967	20	400	0.098	8.00
HD 2404	40	1325	0.099	45.00
HD 2281	30	800	0.116	32.00
HD- 2135	40	1550	0.137	55.00
HD 2687	40	1850	0.133	65.00
HW 2004	40	950	0.145	32.00
HW 2045	40	850	0.076	32.00
JAUW 584	50	1000	0.114	50.00
KALYAN SONA	45	1325	0.099	45.00
K 65	25	1025	0.127	32.00
LAL BAHADUR	25	275	0.092	10.00
NP 715	40	1000	0.127	32.00
PBW 154	40	700	0.075	30.00
PBW 175	31	1005	0.124	24.80
PBW 343	90	2325	0.135	70.00
PBW 443	65	1800	0.127	45.00
PBW 550	65	1850	0.165	65.00
PBW 373	65	2125	0.123	60.00
RAJ 821	40	400	0.098	8.00
RSP 561	65	1375	0.118	65.00
RAJ 1482	80	2225	0.069	65.00
RAJ 3077	15	225	0.041	6.00
RAJ 3765	70	1800	0.124	65.00
SONALIKA	40	1175.25	0.078	28.00
SARBATI SONORA	40	1150	0.105	32.00
SONORA 64	45	950	0.145	32.00
UP 2425	31	1005	0.124	24.80
UP 1109	40	1850	0.133	30.00
UP 2121	30	745	0.114	10.00
VL 404	40	850	0.116	65.00
VL 616	20	800	0.098	32.00
VL 804	25	625	0.063	10.00
VL 421	35	865	0.114	10.00
WH 542	45	1350	0.075	35.00
WH 533	25	275	0.092	10.00
WH 711	40	1575	0.143	30.00
WH 1105	40	1235	0.118	65.00

* FRS= Final rust severity, ** Area Under Rust Progress Curve, ***Coefficient of infection, ****r =infection rate

65%) and high (66-90%) of FRS. Fifteen varieties viz., VL 616, HD 2329, VL 804, HD 2307, HD 2967, RAJ 3077, HD 2864, LAL BAHADUR, UP 2425, WH 533, UP2121, PBW 175, K 65, HD 2281 and VL 421 exhibited low FRS (1-35%), while thirty-one varieties (RAJ 821, RSP 561, JAUW 584, HS 240, PBW 550, HD 2824, WH 542, KALYAN SONA, CPAN 3004, SONALIKA, SARBATI SONORA, DBW 17, HW 2004, DBW16, DPW 621-50, DWR 16, NP 715, GW 496, UP- 1109, HD 2135, WH 711, WH 1105, PBW 443, VL 404, PBW 154, SONORA 64, HD 2987, DURGAPUR 65, HW 2045, PBW 373 and HD2404) having moderate FRS (36-65%), whereas four varieties viz., AGRA LOCAL, RAJ 1482, RAJ 3765 and PBW 343 showed high FRS of 61-90%. Data further showed that thirteen varieties viz., RAJ 821, VL 616, HD 2329, VL 804, HD 2307, RAJ 3077, HD 2864, HD 2967, LAL BAHADUR, WH 533, PBW 154, UP 2121 and HD 2281) were grouped together having value of AURPC range (0-800), while 25 varieties (RSP 561, JAUW 584, HS 240, HD 2824, WH 542, KALYAN SONA, CPAN 3004, SONALIKA, SARBATI SONORA, HW 2004, DBW 16, DPW 621-50, UP 2425, DWR 16, NP 715, HD 2135, WH 711, WH 1105, VL 404, SONORA 64, PBW 175, HW 2045, K 65, HD 2404 and VL 421) exhibited AURPC value of 800-1600, whereas twelve varieties (RAJ 1482, PBW 550, AGRA LOCAL, DBW 17, GW 496, UP 1109, PBW 443, DURGAPUR 65, RAJ 3765, PBW 373 and PBW 343 showed high value of AURPC (1600-2400). Data on disease severity and host reaction was combined to calculate coefficient of Infection (CI) which showed that twenty eight varieties (RAJ 821, VL 616, HD 2329, HS 240, VL 804, HD 2307, WH 542, RAJ 3077, HD 2967, CPAN 3004, SONALIKA, SARBATI SONORA, LAL BAHADUR, HW 2004, DBW 16, UP 2425, NP 715, WH 533, UP 1109, WH 711, PBW 154, SONORA 64, UP 2121, PBW 175, HW 2045, K 65, HD 2281 and VL 421) showed CI value of 1-35, while 22 varieties (RSP 561, RAJ 1482, JAUW 584, PBW 550, AGRA LOCAL, HD 2824, KALYAN SONA, HD 2864, PBW 343, DBW 17, DPW 621-50, DWR 16, GW 496, HD 2135, WH 1105, PBW 443, VL 404, HD 2687, DURGAPUR 65, RAJ 3765, PBW 373 and HD 2404) showed CI value of 36-70. Infection rate 'r' of 50 varieties varied from 0.020-0.169. Among them, RAJ 3077 exhibited 'r' of 0.041, whereas seventeen varieties (RAJ 821, VL 616, HD 2329, RAJ 1482, VL 804, WH 542, KALYAN SONA, HD 2864, HD 2967, SONALIKA, LAL BAHADUR, DBW 16, DPW 621-50, WH533, PBW 154, HW 2045 and HD2404 showed value of 'r' between 0.050- 0.100, while 32 varieties (RSP 561, JAUW 1482, HS 240, PBW 550, AGRA LOCAL, HD 2824, HD 2307, PBW 343, CPAN 3004, SARBATI SONORA, DBW 17, HW 2004, UP 2425, DWR 16, NP 715, GW 496, UP 1109, HD 2135, WH 711, WH 1105, PBW 443, VL 404, SONORA 64, HD 2687, DURGAPUR 65, UP 2121, PBW 175, RAJ 3765, K 65, PBW 373, HD 2281 and VL 421) exhibited value of 'r' between 0.100-1.70. Ali *et al.* (2008) observed a strong association between the coefficient of infection (CI) and final rust severity (FRS) and concluded that wheat varieties having lower FRS value exhibiting slow rusting (partial resistance) against the *Puccinia striiformis*. Dehghani and

Moghaddam (2004) also showed that wheat lines, which had low values of FRS at adult plant stage, exhibited durable resistance (slow rusting or high-temperature adult plant resistance) for a long time, even if pathogen mutated because it is governed by minor genes. Parlevliet (1988) recorded that breeding lines with low value of AURPC, r AURPC, CI and 'r' were expected to possess genes that conferred partial resistance.

Molecular characterization by using alleles - specific primer

Twenty three varieties (DWR 16, PBW 343, VL 616, DBW 16, WH 711, GW 496, UP 2121, HD 2687, PBW 175, HD 2135, HS 240, HW 2045, RAJ 1482, PBW 550, UP 2425, PBW 443, WH 1105, SONALIKA, PBW 154, HD2967, RAJ 3077, RAJ 3765 and HD 2864) amplified a band of 517 bp while remaining varieties (SONARA 64, HD 2281, DURGAPUR 65, WH 542, VL 421, K 65, CPAN 3004, RSP 561, HD 2307, SARBATI SONORA, HD 2404, DBW 621-50, PBW 373, JAUW 584, WH 533, RAJ 821, UP 1109, VL 804, LAL BAHADUR, DBW 17, HW 2004, VL 404, HD 2824, AGRA LOCAL, NP 715, KALYAN SONA and HD 2329) didn't amplify any band (Fig. 1). The amplification of a band of 517 bp indicated the presence of *Yr18* gene which is in agreement with Lagudah *et al.* (2006). Locus *Lr34/Yr18* confers partial and durable resistance against the multiple fungal pathogens viz., leaf rust, stripe rust and powdery mildew and showed that alleles of resistant and susceptible cultivars differed by only three sequence polymorphism and characterized as diagnostic molecular marker that would assist the selection for durable multiple-pathogen resistance (Pathan *et al.*, 2007; Lagudah *et al.*, 2009).

Effect of foliar application of various fungicides on the severity of stripe rust in different varieties of wheat

Data in Table 2 revealed that during 2013-14 and 2014-15 all the fungicides were significantly effective reducing the severity of stripe rust of wheat as compared to untreated controls (51.25%). The fungicide Amistar Top was most effective having the minimum mean disease severity in all the test varieties viz., HD 2967 (7.41%), DPW 621-50 (12.75%), RSP 561 (13.71%) and PBW 343 (19.35) which was followed by Quadrix (8.10, 17.33, 18.63, 28.71%), Score (11.05, 23.63, 25.45, 39.34%), Folicur (12.19, 26.33, 28.80, 45.30%) and Tilt (13.46, 30.19, 33.59 and 52.65%) in wheat varieties HD 2967, RSP 561, DPW 621-50 and PBW 343, during both the cropping season. Application of Triadimefon, Fenarimol, Benomyl, and Oxycarboxin was effective to control the stripe and leaf rust of wheat (Wanyera *et al.*, 2010). Ahanger *et al.* (2014) recorded that Quadrix (0.1%) proved most effective in reducing disease severity of stripe rust in susceptible varieties as compared to moderate resistant varieties. Loughman *et al.* (2005) observed that foliar fungicides achieved economic control only when applied at an early stage of disease initiation as early intervention reduced damage and ensured translocation of nutrients for proper grain filling. Han *et*

Fig. 1. PCR amplification of 50 wheat varieties with *cssr1* showing amplification of 517 bp band. M – Ladder, Samples - 1 (SONARA 64), 2 (DWR 16), 3 (PBW 343), 4 (HD 2281), 5 (DURGAPUR 65), 6 (VL 616), 7 (DBW 16), 8 (WH 542), 9 (WH 711), 10 (GW 496), 11 (VL 421), 12 (K 65), 13 (UP 2121), 14 (cPAN 3004), 15 (RSP 561), 16 (HD 2687), 17 (HD 2307), 18 (SARBATI SONORA), 19 (PBW 175), 20 (HD 2404), 21 (HD 2135), 22 (DBW 621-50), 23 (HS 240), 24 (PBW 373), 25 (JAUW 584), 26 (HW 2045), 27 (RAJ 1482), 28 (WH 533), 29 (RAJ 821), 30 (UP 1109), 31 (PBW 550), 32 (UP 2425), 33 (VL 804), 34 (LAL BAHADUR), 35 (DBW 17), 36 (HW 2004), 37 (VL 404), 38 (HD 2824), 39 (PBW 443), 40 (WH 1105), 41 (SONALIKA), 42 (PBW 154), 43 (AGRA LOCAL), 44 (NP 715), 45 (HD 2967), 46 (RAJ 3077), 47 (RAJ 3765), 48 (KALYAN SONA), 49 (HD 2329), 50 (HD 2864)

al. (2006) observed that fungicide, Tebuconazole, not only inhibited the development of *P. striiformis* in wheat leaves but also accompanied several morphological and structural changes in the hyphae and haustorium and increased vacuolation, irregular cell wall thickening and necrosis or degeneration of cytoplasm. They further recorded enhanced biochemical host defense reactions in the infected host by the accumulation of the two enzymes (chitin and β -1,3-glucanase) and lignin after fungicidal treatment.

Effect of foliar application of various fungicides on yield of different varieties of wheat

The data presented in Table 3 revealed that in HD 2967 maximum yield of 45.64 q/ha was recorded by application of Amistar Top during both the cropping season which was significantly superior to Quadrix (39.47q/ha) followed by Score (38.45q/ha), Folicur (36.72q/ha) and Tilt (32.67q/ha), whereas in untreated control mean grain yield was (30.28q/ha). Similarly, in other varieties viz., RSP 561, DPW 621-50 and PBW 343, application of Amistar Top resulted in maximum mean yield of (38.50, 32.86 and 25.52 q/ha) followed by Quadrix with (37.48, 30.41 and 23.93 q/ha), Score with (36.65, 28.40 and 22.77 q/ha), Folicur (35.40, 29.38 and 21.58 q/ha) and Tilt (31.49, 27.82 and 20.69 q/ha), respectively. The result was in conformity with the findings of Lopez *et al.* (2014) who recorded increased yield in wheat cultivars by the

application of Tebuconazole. Singh *et al.* (2016) recorded that stripe rust was controlled by azoles and strobilurin group of fungicides, which also increased the grain yield and quality.

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