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Identification of yellow rust resistance sources in advanced breeding lines of barley (*Hordeum vulgare* L.)

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Abstract

Yellow rust of barley caused by *Puccinia striiformis* f. sp. *hordei (Psh),* is an important disease in barley growing regions of India and worldwide. For identifying adult plant stage resistance to yellow rust, a set of 29 barley advanced breeding lines was tested at five locations, Durgapura, Jammu, Ludhiana, Bajaura and Karnal during 2016-17 and 2017-18. The seedling resistance test was conducted on 27 barley advanced breeding lines against seven *Psh* pathotypes separately under controlled conditions at ICAR-IIWBR, Shimla during 2017-18. As consequences, seven lines (DWRFB10, DWRFB12, DWRFB14, DWRFB15, DWRFB19, DWRFB20 and DWRFB28) were found immune to highly resistant against yellow rust at adult plant stage across the locations. Similarly, seven advanced breeding lines *viz*., DWRFB11, DWRFB12, DWRFB13, DWRFB14, DWRFB19, DWRFB20 and DWRFB27, were found resistant to yellow rust at seedling stage. On the basis of APR and SRT, four advanced barley breeding lines, DWRFB12, DWRFB14, DWRFB19 and DWRFB20 were highly resistant to yellow rust (nearly immune) both at seedling and adult-plant stages.

Keywords: *Hordeum vulgare*, barley, yellow rust, barley diseases, genetic resistance, *Puccinia striiformis*

1. Introduction

Barley (*Hordeum vugare* L.) is one of the founder crops of the world agriculture. According to the acreage and production, barley occupied fourth ranks after wheat, rice and maize at world level during 2009 (Pandey *et al.,* 2009). It is cultivated in varying agro-climatic conditions such as rainfed areas, dry lands, saline-alkaline soils, flood prone, marginal and coastal areas in the world. Hence, barley is considered an important crop for resource poor farmers in many developing countries. In India, barley is grown about 0.693 million hectares area with 1.79 million tonnes production and a productivity of 2580 kg/ha (Anonymous, 2017). It is consumed in varied forms including animal feed, human food and for malting and brewing in industry. This crop is considered as poor man's crop in India because of its low input requirement and better adaptability in the harsh environments (Verma *et al.*, 2012).

Barley suffers from several diseases responsible for heavy reduction in yield and grain quality. Out of them, yellow rust is an important disease of barley, caused by *Puccinia striiformis* Westend. f. sp. *hordei* Eriks. & Henn. (Psh). Generally, it occurs in the barley growing areas of the northern India and cooler parts of many countries of world (Prashar *et al.,* 2014).The disease initiates appearing in the plains during mid-December to beginning of January and thrives well

under the cold conditions (Prakash and Verma, 2009). It is also prevalent and destructive at higher altitude in Ladakh region of India (Vaish *et al.*, 2011). The incidence of yellow rust may create serious problems by growing susceptible varieties and as a consequence in heavy yield losses. Yield losses caused by *P. striiformis* f. sp. *hordei* were estimated up to 60% (Park *et al.*, 2007). Severe epidemics of the disease have been observed in north-western and central European countries, India, Bangladesh, Nepal, China and Japan (Chen *et al.*, 1995). Early incidence of yellow rust disease can cause heavy damage to barley crop in Indian environmental conditions and sometimes, it prevents emergence of heads or grain formation (Prakash and Verma, 2009). The yield losses may be reduced by controlling the disease through application of the fungicides (Marshall and Sutton, 1995). However, it is not considered as an economical and environmental friendly approach to control the disease. Therefore, development of resistant varieties against yellow rust becomes inevitable. The current study was conducted to identify the resistant sources to yellow rust disease in twenty nine advanced breeding lines at adult-plant stage and in twenty seven lines at seedling stage.

2. Materials and methods

2.1. Adult Plant Resistance Test

The experimental material comprised of total 29 advanced breeding lines of feed barley which were evaluated against yellow rust disease at five different hot spot locations (Durgapura, Ludhiana, Bajaura, Jammu and Karnal) during 2016-17 and 2017-18 seasons under artificial rust epiphytotic conditions (Table 1). The field experiments were conducted in the month of November of both consecutive years. Each test line was sown in single row of 1.0 m length and a distance of 30 cm was maintained between the rows. A susceptible check (Bilara-2) was raised before the first and after the last test lines. Susceptible infector lines (mixture of susceptible cultivars BL2, RD31, RS6, Jyoti and RD2035) were sown on all four sides of the test lines. The susceptible infector rows were artificially injected with the spore suspension prepared by mixing of uredospores of five *Psh* pathotypes *i.e.* 24(0S0-1), 57(0S0), G (4S0), M (1S0) & Q (5S0), just before early tillering stage (Zadoks GS 10-19). Additionally, in the main field, infectors were also inoculated 3-4 times by spraying from tillering to flag leaf stage to trigger the development of rust epidemics. Five irrigations were applied at different growth stages to the crop for maintaining the moisture and disease development. The observations on yellow rust were recorded by combining severity (percent leaf area covered by rust) and response (infection type). The plants were scored when the disease appeared completely on the infector rows. The scoring for yellow rust was done using the modified Cobb's scale (Peterson *et al.*, 1948). The host response in the field was scored as Resistant (R) = no uredia present; Moderately Resistant (MR) = small uredia with slight sporulation; Moderately Susceptible (MS) = medium sized uredia with moderate to heavy sporulation and Susceptibility (S) = large uredia with abundant sporulation. The disease severity and host response data were combined into a single value

Table1. Advanced breeding lines of barley derived by crossing between indigenous and exotic genotypes for APR and SRT study

Genotype	Pedigree	Genotype	Pedigree
DWRFB1	RD 2715/BCU 8	DWRFB16	IBYT-HI-9 (2010-11)/BCU 6631
DWRFB2	RD 2715/BCU 8	DWRFB17	IBYT-HI-1 (2010-11)/BCU 2881
DWRFB3	RD 2715/BCU 1549	DWRFB18	IBYT-HI-1 (2010-11)/BCU2881
DWRFB4	RD 2715/BCU 1549	DWRFB19	CDC MANLEY/BCU 2881
DWRFB5	P L 426/ EIBON 18	DWRFB20	CDC MANLEY/BCU 2881
DWRFB6	P L 426/EIBON 19	DWRFB21	RD 2052/RD 2092
DWRFB7	P L 426/EIBON 19	DWRFB22	DL 456/RD 2592
DWRFB8	P L 426/EIBON 19	DWRFB23	RD 2035/BCU 6038
DWRFB9	P L 426/IBYT-LRA-C1	DWRFB24	VM 150/BCU 8
DWRFB10	DWR 83/EIBON 18	DWRFB25	VM 150/BCU 8
DWRFB11	DWR 83/EIBON 18	DWRFB26	VM 150/BH 902
DWRFB12	DWR 83/EIBON 18	DWRFB27	RD 2552/IND 253
DWRFB13	DWR 83/EIBON 18	DWRFB28	IBON-(2015)-59
DWRFB14	DWR 83/EIBON 19	DWRFB29	IBON-(2015)-49
DWRFB15	DWR 83/EIBON 19		

called the coefficient of infection (CI). The coefficient of infection was estimated by multiplying of disease severity (DS) and constant values of infection type (IF). The constant values for infection types were immune=0.0, R=0.2, MR=0.4, M=0.6, MS=0.8 and S=1.0 (Stubbs *et al.*, 1986). The average coefficient of infection (ACI) was calculated. The categorization of advanced lines into resistance and susceptible was done based on ACI values (Sajid *et al.*, 2009). The advanced lines with pooled ACI value 0 or <1, were considered highly resistant (nearly immune). The lines with ACI values of 0-5 were considered to possess strong seedling resistance instead of adult-plant resistance. However, ACI values 5-20 were considered as high adult-plant resistance, 21- 40 as moderate and 41-60 as low adult-plant resistance. Advanced lines with ACI values >60 were considered susceptible or no adult-plant resistance.

2.2. Seedling resistance test

The seedling resistance test was performed on twenty seven barley advanced breeding lines during 2017-18 at Regional Station, IIWBR, Flowerdale, Shimla, using seven pathotypes (6S0, 7S0, G, M, 24, 57 and Q) separately under controlled conditions. Barley lines were grown in aluminium bread pans (29 x 12 x 7 cm) containing a mixture of fine loam and farm yard manure (3:1). These trays were sufficiently large to accommodate 18 lines and a susceptible check (Bilara-2). For each barley line, about 4-5 seeds were sown in hills. One-week old seedlings were inoculated using a glass atomizer containing 10 mg spores of an individual *Psh* pathotype suspended in 1.0 ml light grade mineral oil (Soltrol 170®). Thereafter, inoculated barley lines were sprayed with a fine mist of water and kept in a moist chamber (RH >80%) at 12±2°C for 48 hours. Subsequently, they were transferred on to the green house benches where appropriate temperature (16±2°C), relative humidity (60-80%) and illumination (about 15,000 lx for 12 hours) were maintained (Gangwar *et al.,* 2018). The data were recorded on reaction type of these lines against each pathotype at 16-18 days post-inoculation (Nayar *et al.*, 1997).

3. Results and discussion

Yellow rust is one of the major biotic stresses in barley production. Yellow rust pathogen is obligate parasite of crop plants and evolves frequently in many distinct physiologic races or pathotypes. New pathotypes spread rapidly because of high reproductive rate and airborne nature (Duveiller *et al.*, 2007; Duplessis *et al.*, 2011). Pathotype 6S0 and 7S0 are newly emerged and virulent on both wheat and barley hosts. Pathotype 1S0 (M) is predominant and widely distributed across the North Indian states followed by pathotype 0S0 (57) (Prashar *et al.*, 2014; Gangwar *et al.*, 2016, Gangwar *et al.*, 2019). The major resistance genes are generally overcome by new virulent pathotypes and thus, identification and exploitation of new sources of resistance has become essential for sustainable rust resistance breeding program. In this study, seven advanced breeding lines (DWRFB11, DWRFB12, DWRFB13, DWRFB14, DWRFB19, DWRFB20 and DWRFB27) were found resistant (IT: 0;) to all *Psh* pathotypes at seedling stage. On the contrary, 12 advanced breeding lines were susceptible (IT: 3, 3$^+$) to all the pathotypes. The infection types (ITs) on advanced breeding lines at seedling stage (all-stage resistance) are presented in Table 2. The identified resistant sources to yellow rust

Table 2. Seedling Resistance Test (SRT) of advanced breeding lines of barley to individual race of *Puccinia striiformis* f.sp. *hordei*

Genotype	Reaction* to *Psh* races						
	6S0	7S0	G	M	24	57	Q
DWRFB1	33$^+$	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$
DWRFB2	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$
DWRFB3	3$^+$	33$^+$	3	2$^+$	3$^+$	3$^+$	2$^-$
DWRFB4	3$^+$	3$^+$	3	3$^+$	3$^+$	3$^+$	2$^-$
DWRFB5	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	3
DWRFB6	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	3
DWRFB7	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$
DWRFB8	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$
DWRFB9	33$^+$	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	0;
DWRFB10	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$
DWRFB11	0;	0;	0;	0;	0;	0;	0;
DWRFB12	0;	0;	0;	0;	0;	0;	0;
DWRFB13	0;	0;	0;	0;	0;	0;	0;
DWRFB14	0;	0;	0;	0;	0;	0;	0;
DWRFB15	0;	3$^+$	0;	0;	0;	0;	0;
DWRFB16	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$
DWRFB17	3	0;	2$^-$	0;	3	2	0;
DWRFB18	3	3	2	0;	0;	3	0;
DWRFB19	0;	0;	0;	0;	0;	0;	0;
DWRFB20	0;	0;	0;	0;	0;	0;	0;
DWRFB21	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$
DWRFB22	3	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$
DWRFB23	33$^+$	3$^-$	3$^+$	3$^-$	3$^+$	33$^+$	2$^-$
DWRFB24	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	2$^-$
DWRFB25	3$^+$	3	3$^+$	3	3$^+$	3$^+$	3$^-$
DWRFB26	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$
DWRFB27	0;	0;	0;	0;	0;	0;	0;
BHS 352(C)	3$^-$	-	3	3$^+$	3	3$^+$	2$^-$
Gitanjali (c)	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$
Karan 16	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$
NDB 943	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$	3$^+$

*3$^+$=susceptible, 3, 3$^-$ = moderately susceptible, 2, 2$^+$ = moderately resistant, 0;/;/1/2$^-$ = Resistant

Table3. Field Response of advanced barley breeding lines to yellow rust reactions

Genotype	IBDSN 2016-17						IBDSN 2017-18						Pooled ACI value
	Durgapura	Jammu	Ludhiana	Bajaura	Karnal	ACI	Durgapura	Jammu	Ludhiana	Bajaura	Karnal	ACI	
DWRFB1	100S	40S	5S	80S	60S	57.00	80S	10S	20S	60S	40S	42.00	49.50
DWRFB2	80S	40S	10S	80S	60S	54.00	80S	10S	20S	60S	40S	42.00	48.00
DWRFB3	30MS	10S	0	80S	0	22.80	20S	0	10S	60S	5MR	18.40	20.60
DWRFB4	40S	0	0	80S	10S	26.00	40S	0	20S	60S	10S	26.00	26.00
DWRFB5	60S	40S	5S	60S	40S	41.00	60S	10S	40S	60S	10MS	35.60	38.30
DWRFB6	60S	20S	5S	60S	40S	37.00	40S	5R	40S	40S	20S	28.20	32.60
DWRFB7	60S	20S	5S	60S	40S	37.00	40S	0	20S	60S	20S	28.00	32.50
DWRFB8	30S	20S	5S	0	60S	23.00	20S	5R	20S	40S	20S	20.20	21.60
DWRFB9	60S	5MS	10S	80S	40S	38.80	80S	20S	40S	30S	20S	38.00	38.40
DWRFB10	0	0	0	0	20S	4.00	0	TMS	0	0	0	0.16	2.08
DWRFB11	0	0	0	80S	0	16.00	0	0	0	0	0	0.00	8.00
DWRFB12	0	5MS	0	0	0	0.80	0	0	0	0	0	0.00	0.40
DWRFB13	0	5MS	0	60S	0	12.80	0	10S	0	0	0	2.00	7.40
DWRFB14	0	0	0	0	0	0.00	0	0	0	0	0	0.00	0.00
DWRFB15	0	TMS	0	0	0	0.16	0	0	0	0	20S	4.00	2.08
DWRFB16	80S	5S	5S	80S	40S	42.00	80S	0	40S	60S	40S	44.00	43.00
DWRFB17	0	20S	0	60S	0	16.00	5R	20S	5MS	40S	0	13.00	14.50
DWRFB18	5MS	5MS	0	80S	0	17.60	10S	0	5S	40S	5MR	11.40	14.50
DWRFB19	0	0	0	0	0	0.00	TR	TR	TR	0	0	0.12	0.06
DWRFB20	0	0	0	0	0	0.00	0	TR	0	0	0	0.04	0.02
DWRFB21	100S	5S	20S	40S	40S	41.00	100S	0	10S	0	20S	26.00	33.50
DWRFB22	60S	NIL	10S	40S	40S	37.50	40S	20S	20S	40S	20S	28.00	32.75
DWRFB23	30S	NIL	0	0	0	7.50	20S	20S	10S	40S	10MS	19.60	13.55
DWRFB24	60S	NIL	5S	40S	40S	36.25	20S	0	10S	60S	10MS	19.60	27.92
DWRFB25	60S	0	5S	40S	20S	25.00	20S	20S	10S	60S	20S	26.00	25.50
DWRFB26	60S	10S	10S	60S	40S	36.00	20S	0	10S	60S	40S	26.00	31.00
DWRFB27	0	5MS	0	20S	0	4.80	0	TMS	0	40S	0	8.16	6.48
DWRFB28	10MS	0	5S	0	0	2.60	0	TR	0	0	0	0.04	1.32
DWRFB29	15S	0	0	15S	10MS	7.60	10S	TR	5S	0	20S	7.04	7.32
Infector	100S	60S	60S	80S	80S	76.0	100S	60S	60S	80S	60S	72.00	74.00

IBDSN- Initial Barley Disease Screening Nursery; ACI- Average Coefficient of Infection; R- Resistant; TR-Traces to Resistant; MR-Moderately Resistant; TMR-Traces to Moderately Resistant; MS-Moderately Susceptible; S-Susceptible

in the present study can be exploited as donor parent in barley breeding program.

The adult plant resistance (APR) is usually expressed at adult plant stage and also referred as partial resistance, horizontal resistance, durable resistance and non-race specific. Adult plant resistance is believed to be governed by several additive minor genes and generally more durable than seedling or all-stage resistance (Singh and Rajaram, 1992). On the basis of pooled ACI value (<5.00), lines, DWRFB10, DWRFB12, DWRFB14, DWRFB15, DWRFB19, DWRFB20, and DWRFB28 revealed highly resistant reaction to yellow rust across the locations at adult-plant stage (Table 3). The ACI value, in the range of 0-5, is considered to possess strong seedling resistance instead of adult-plant resistance. Therefore, it appears that these lines might have strong all-stage resistance gene combined with few miner genes. The pooled ACI value of advanced breeding lines, DWRFB11, DWRFB13, DWRFB17, DWRFB18, DWRFB23, DWRFB27 and DWRFB29

was in between 5 and 20. These lines supposed to possess high adult-plant resistance. None advanced line was categorised as susceptible with ACI >60. Earlier, 336 barley genotypes from ICARDA were evaluated against Indian *Psh* pathotype both at seedling and adult-plant stages. Of the total, 12 barley genotypes (ARAMIR/COSSACK, Astrix, C8806, C9430, CLE202, Gold, Gull, Isaria, Lechtaler, Piroline, Stirling, and Trumpf) were resistant to six *Psh* pathotypes (24, 57, M, G, Q and 6S0) both at the seedling and adult-plant stages (Verma *et al.*, 2018; Gyawali *et al.*, 2018).

In conclusion, four advanced barley breeding lines, DWRFB12, DWRFB14, DWRFB19 and DWRFB20 were identified as nearly immune to yellow rust both at seedling and adult-plant stages. These lines have been developed by crossing indigenous and exotic genotypes and more adaptable to local environmental conditions. Therefore, identified resistant sources can be exploited directly or used in barley breeding program for developing yellow rust resistant varieties.

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