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Characterization of exotic barley genotypes for barley yellow dwarf virus in District Peshawar, Khyber Pakhtunkhwa, Pakistan

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Abstract

Barley yellow dwarf disease (BYDD) is a destructive viral disease of cereals. Globally significant yield losses were recorded in cereal crops; BYDD losses in barley ranged from 25 to 30%. In the Province Khyber Pakhtunkhwa, Pakistan sever yield losses were also recorded in cereal crops. Barley yellow dwarf virus (BYDV) prevalence, incidence, symptoms, severity and resistance were studied in a set of 26 exotic barley genotypes along with two local barley genotypes (Sanober-96 and Ajj) over two years in district Peshawar, Khyber Pakhtunkhwa, Pakistan. During the study period barley/cereal yellow dwarf virus (B/CYDV) symptoms were consistently and uniformly observed. Variations observed among phenotypic assessments with regards to percent Barley yellow dwarf disease (BYDD) severity and incidence (infected tillers/plot) was found among the genotypes. The highest BYDD severity (60%) and incidence (65%) were recorded during barley growing season 2017. A significant interaction among genotypes and years was found ($P<0.05$) while the interaction among genotypes cross years were found non-significant. Six genotypes, B-SA-1, B-SA-10, B-SA-11, B-SA-13, B-SA-19, and B-SA-14, were found resistant to BYDV. On the basis of presence of phenotypic markers "the leaf tip necrosis (LTN)" which is linked with BYDV resistant gene Bdv1. Improvement in cultivation of indigenous and exotic wheat and barley varieties with BYDV resistant genes could be possible to combat with this serious problem in Khyber Pakhtunkhwa province of Pakistan.

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Introduction

Yellow dwarf viruses (*YDVs*) are one of the significant casual factors of diseases in cereal crops, yellow dwarf viruses diseases (*YDVs*) are categorized as Barley yellow dwarf viruses (*BYDs*), Cereal yellow dwarf viruses (*CYDVs*), and Wheat yellow dwarf virus (*WYDV*).Yellow dwarf viruses have been conformed in both agriculturally important cereal crops and non-crop grasses globally (Hesler 2005; Hawkes and Jones, 2005; Kumari *et al*., 2006; Power *et al*., 2011; Siddiqui *et al*., 2012; Jarosova *et al*., 2013).

The *YDVs* belong to the *Luteoviridae* family of viruses. In Pakistan *BYD* was first noticed on the basis of symptoms in 1964 in wheat crop, near pak-afghan border (Aslam and Ahmad, 1987). *BYD* infectivity can direct to phloem destruction and damage. Stunted development (dwarf emergence) and changes in color of leaf alongside the vascular tissues, particularly leaf tip (Kosova *et al*., 2008).

Cool temperatures and high light concentration ranging from 15 to18°C have been establish to support appearance of *BYD* symptoms (Darcy and Domier, 2000). Strain-specific antibodies are well-known for six of the B/CYDVs*: BYD-RMV, BYD-MAV, BYD-PAV, BYD-SGV, BYD-GAV* and *CYD-RPV* (Wang *et al*., 2001; Lister and Rochow, 1978). Recently nucleic acid-based diagnostics practice are developed and has been revolutionized the discovery of phytopathogens (Vincelli and Tisserat, 2008; Elnifro *et al*., 2000).

The real time polymerase chain reaction (RT-PCR) technique is very efficient for primers amplification (Malmstrom and Shu, 2004; Zhang *et al.,* 2009) or other positive, single stranded RNA (+ssRNA) viruses as luteoviruses responsible for the diseases on the same hosts (Viswanathan *et al*., 2010) in both multiplex and monoplex RT-PCR. Furthermore, RT-PCR is capable to detect these viruses in their aphid vectors (Liu *et al*., 2007; Li *et al*., 2001).

To combat with BYDD, cultural practices like the sowing date or the management of aphids by using of aphicides are considerably useful to the decline of B/CYDV disease; while the use of resistant germplasm is generally regarded as most effective and eco-friendly (Miller and Rasochova, 1997). There is no known complete resistance to B/CYDV in barley (Ordon *et al*., 2009). Growing of host plant resistance through genetic engineering is an additional promising choice to combat the problem (Shah *et al*., 2012). Thus characterization to BYDD in indigenous and exotic germplasm is crucial for genetic improvement of barley to avoid economic losses due to BYDD (Ford *et al*., 1998).

The main objectives of the current study are to identify barley yellow dwarf resistant Genotypes and effect of temperature on barley genotypes sown in district Peshawar of Province Khyber Pakhtunkhwa, Pakistan.

Materials and methods

The present study was carried out to describe resistance phenotype against barley yellow dwarf disease (BYDD) for exotic and local barley germplasm. Field survey made it possible to monitor symptoms expressions and aphid infestations, and to sample leaves for molecular analysis.

The study was carried out during barley growing seasons 2015-2017 and was performed at the Institute of Biotechnology and Genetic Engineering (IBGE), Agriculture University, Peshawar, Pakistan.

*Selection of genotypes and location for field trials*Twenty-six exotic barley genotypes introduced from Europe through Global Rust Reference Centre (GRRC, Arhus University, Denmark) along with two local genotypes (Sanober-96 and Ajj) were selected for the study. These 28 genotypes were sown at district Peshawar (at IBGE Research Farm, the University of Agriculture) (Fig.1).

A map of Pakistan with the different provinces is presented at the bottom right corner of the figure. KP province and Fata are colored in green and blue, respectively. The location used to grow barley in the experiments is presented with red circle on the map.

Fig. 1. Map of Khyberpakhtunkhwa (KP) province and Fata (FATA) of Pakistan.

Experimental design for field plots

Seeds from all genotypes were sown in undersized contiguous plots according to randomized complete block design (RCBD) with three replications. Each plot corresponds to four rows with 1 m row length, 0.3 m row to row and 0.6 m plot-to-plot distance.

Variability in viral prevalence and resistance in exotic germplasm

The presence of virus in the field was monitored based on symptom expression during both periods of the growing season. Field surveys were scheduled from mid-December to the end of March. This procedure made it possible to record expression of first disease symptoms (mainly yellowing and reddening) and to collect data on the evolution of symptoms during the three-months monitored period. Symptomatology based incidence and severity of yellow dwarf disease were taken according to the 0-9 scale as described by Carrigan *et al.* (1981) where 0 stands for plants with no symptom and 9 for plants with maximum chlorosis and necrosis. At least three data scoring were made for the trial. The sanitary status of plots in the locations was assessed through comparison of maximum and minimum symptom intensity, and the percentage of symptomatic plants observed at a given location. The average barley yellow dwarf disease incidence of the three intervals was used for analysis and input for disease advancement and area under disease progress curve (AUDPC) resolve. The AUDPC standards were planned using the given formula:

$$AUDPC = \sum^{n-1} 0.5(t_i + 1 - t_i)(x_i + 1 + x_i)$$

Where 'n' is the total number of remarks, t_i is the time (duration after sowing) at the $i^{th,}$ assessment and x_i is the growing infection severity expressed in a quantity at the x_i assessment.

Data analysis and interpretation

All parameters were compiled in MS excel and modified as input files for various software's, for further analysis. Statistical analyses of the data were

carried out through analysis of variance (ANOVA) technique appropriate for randomized complete block design (RCBD) using computer software MSTATEC for all parameters.

Results and discussion

BYDV occurrence and percent severity

The tested exotic barley genotypes showed variation in barley yellow dwarf disease occurrence. The disease pressure was assisted using infected tillers per plot and % barley yellow dwarf disease (BYDD) severity on exotic barley genotypes as well as on two local barley lines (AJJ and sanober-96) (Table 2) and were found that over all the viral prevalence (Fig. 2) was high in 2017 as compared to 2016 for the reason of difference in ecological conditions such as temperature and humidity (Smyrnioudis, 2001; Smyrnioudis, 2000). Similarly, cold environment ranging from 15 to 18 °C is shown to support *BYDD* symptoms appearance as well as magnetize vectors to infected plants (Arcy and Domier, 2000).

Table 1. Weather data recorded during barley growing season 2016 and 2017.

Months	Weather data 2016			Weather data 2017		
	Mean temp	Mean humidity	Total precipitates	Mean temp	Mean humidity	Total precipitates
January	9	63	68	8	52	65.3
February	14	44	82	16	44	92.5
March	22	39	91	28	46.3	80.32
Mean	15	48.6	80.3	17.3	47.4	79.37

Temperature up to 30°C is reported to raise conduction power of incompetent vectors (Arcy and Domier., 2000). Likewise (Bailey, 1995) reported the Drought/low rainfall also favor aphids spread and *BYD* symptoms expressions in host plants.

Table 2. BYDD Mean Area under disease progress curve (AUDPC), % severity, and Resistance class of the tested exotic barley genotypes during 2016 and 2017.

Genotype	Source	Mean AUDPC	Mean % severity	Resistance class
BSA-1	GRRC- Denmark	58	19	R
BSA-2	GRRC- Denmark	264	32	LR
BSA-3	GRRC- Denmark	455	25	LR
BSA-4	GRRC- Denmark	254.5	50	LR
BSA-5	GRRC- Denmark	163	44	LR
BSA-6	GRRC- Denmark	206	48	LR
BSA-7	GRRC- Denmark	191.5	55	MS
BSA-8	GRRC- Denmark	631	70	S
BSA-9	GRRC- Denmark	439.5	40	LR
BSA-10	GRRC- Denmark	17	52	MS
BSA-11	GRRC- Denmark	100	20	R
BSA-12	GRRC- Denmark	129	30	LR
BSA-13	GRRC- Denmark	69.5	45	LR
BSA-14	GRRC- Denmark	58	35	LR
BSA-15	GRRC- Denmark	169	25	LR
BSA-16	GRRC- Denmark	159.5	48	LR
BSA-17	GRRC- Denmark	144.5	39	LR
BSA-18	GRRC- Denmark	141.5	36	LR
BSA-19	GRRC- Denmark	100	15	R
BSA-20	GRRC- Denmark	189	10	R
BSA-21	GRRC- Denmark	163	28	LR
BSA-22	GRRC-Denmark	248	53	MS
BSA-23	GRRC- Denmark	101	45	LR
BSA-24	GRRC- Denmark	120	41	LR
BSA-25	GRRC- Denmark	135	33	LR
BSA-26	GRRC- Denmark	758	85	S
AAJ	CCRI-Pakistan	364.5	18	LR
Sanober-96	CCRI-Pakistan	179	30	LR

The disease incidence was recorded on B-SA-26 (227 infected tillers/plot) during barley growing season 2016-2017, while observed (224 infected tillers/plot) on the same genotype during 2015-2016. Variation in the mean number of infected tillers per plot (Fig. 3) showed a mean incidence to 60 during 2017 while the mean incidence was 45 during 2016 and BYDD symptoms severities (Fig. 4) were also found in which 38% and 65% were observed during the year 2017 and 2016 respectively.

Table 3. Pooled analysis of variance of BYD % severity during 2016 and 2017 at IBGE, Peshawar, Pakistan.

Source	Degrees of freedom	Sum of squares	Mean Square	F Value	P Value
Rep	3	2.0235	0.674	0.1524	
Genotypes (G)	28	367.392	13.121	2.897	0.0002**
Years (Y)	2	433.789	216.89	48.939	0.0001**
G*Y	52	339.752	6.533	1.457	0.0521
Error	150	746.128	4.974		

The variations in BYDD symptoms severity could be due to the following observed three factors (Table 1) that were (i) High mean temperature (ii) low mean precipitation and (iii) low mean % humidity during the peak activity period of *BYDV* vectors. The mean temperature recorded from the month of January to March in the area was 17.3 ˚C during the year 2017 while in barley growing season 2016 the observed mean temperature was low (15 ˚C). Similarly the mean relative humidity and total precipitation recorded for the year 2016 were 48.6 mm and 80.3 mm while for the year 2017 the mean relative humidity and precipitation were 47.4 mm and 79.37 mm respectively.

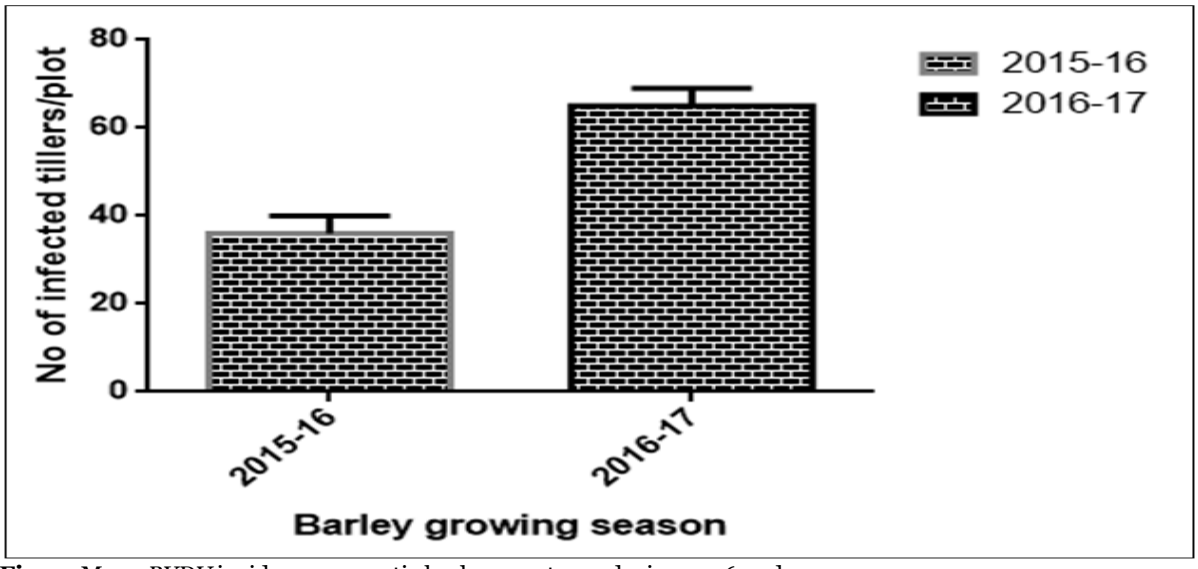

Fig. 2. Mean *BYDV* incidence on exotic barley genotypes during 2016 and 2017.

Aheer *et al.*, (2008) showed the role of relative moisture and warmth in asymmetrical aphid's concentration in wheat crop. Low temperature ranged from 15 to 20 ˚C was showed to support *BYD* symptoms appearance as well as magnetize vectors to infected plants (Arcy and Domier, 2000). Temperature up to 30 °C is reported to raise conduction power of incompetent vectors (Arcy and Domier, 2000; Bailey, 1995). Variation in *BYD* incidence and severity level among wheat varieties, both within and between seasons may occur due to irregular circulation of infected aphid agents and dispersal virus from the area of infection under ordinary field setting. Researchers reported the

dissimilar alteration of a genotype tolerance to BYD in two different growing conditions (Comeau and Jedlinski, 1990). Assuming the BYDD % symptoms severity, as leaf tip necrosis (LTN) (Table 2), the tested barley genotypes were classified into four groups on the basis of mean BYDD symptoms % severity.

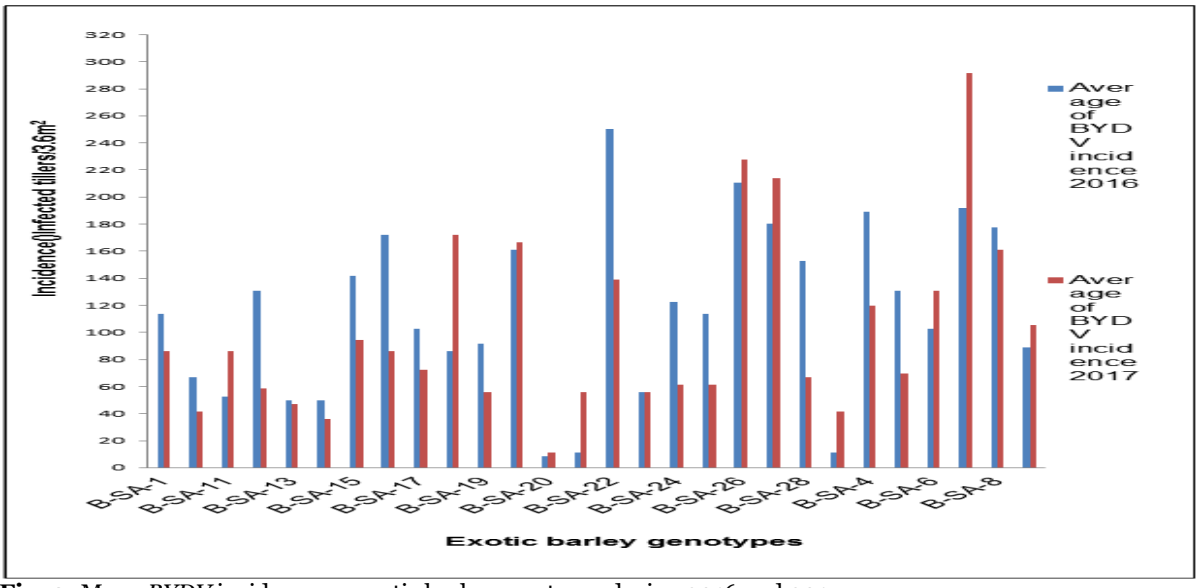

Fig. 3. Mean *BYDV* incidence on exotic barley genotypes during 2016 and 2017.

The group one showed % symptoms severity ranged from 0-20, the group-2 showed % symptoms severity ranged from 21-50%, the group-3 showed % symptoms severity ranged from 51-70%, the group-4 showed % symptoms severity above 70%, were considered as resistant (R), low resistant (LR), moderately susceptible (MS) and susceptible (S) respectively in terms of disease resistance. Four genotypes come in group-1 which were included B-SA-1, B-SA-11, B-SA-19 and B-SA-20, eighteen genotypes comes in group-2, which includes B-SA-2, B-SA-3, B-SA-4, B-SA-5, B-SA-6, B-SA-9, B-SA-12, B-SA-13, B- SA-14, B-SA-15, B-SA-16, B-SA-17, B-SA-18, B-SA-21, B-SA-23, B-SA-24, B-SA-25 and the local barley genotype Sanober-96.

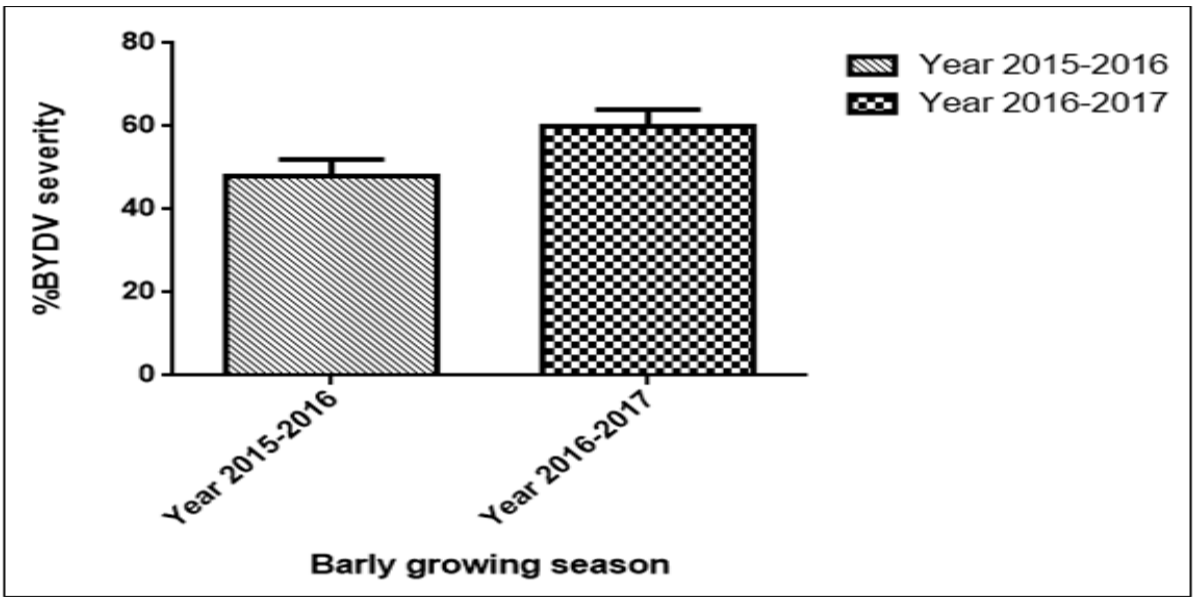

Fig. 4. % *BYDV* Severity on exotic barley genotypes during 2016 and 2017.

Three genotypes including B-SA-7, B-SA-10 and B-SA-22 comes in group-3 whereas two genotypes comes in group-4 which includes B-SA-8 and B-SA-26.the Bdv1 was reported to be resistant to *BYDV* and showed slow yellowing .The gene was first identified by Sing (1993) in Anza wheat. The presence of Bdv1 gene in wheat and barley verities showed slow leaf tip necrosis (Sing, 1992).

The morphological trait, leaf tip necrosis (LTN) used as a phenotypic marker for the detection of barley yellow dwarf virus, Yellow rust and leaf rust. In the present studies the group-1 possess high and the group-2 low frequencies of LTN while the group-3 showed rare frequencies towards phenotypic marker LTN whereas in the group-4 LTN was completely absent respectively. This gene was believed to occur frequently in CIMMYT wheat varieties (Sing, 1993), Similarly LTN conformation for the presence and absence of Bdv1 gene were reported by Dyck (1999) and Shah (2010).

ANOVA was carried out for the tested exotic barley genotypes (Table 3). The *BYDV* % severity was found significantly different ($P<0.01$) for the years and genotypes whereas the interaction of year × genotypes were found non-significant. Performance of individual barley genotypes was presented in Fig. 3.

Based on area under disease progress curve (AUDPC) (Table 2) the tested genotypes were also classified as" resistant" (R) included six genotypes, B-SA-1, B-SA-10, B-SA-11, B-SA-13, B-SA-19, and B-SA-14, "moderately resistant" (MR) included thirteen genotypes, B-SA-5, B-SA-7, B-SA-12, B-SA-15, B-SA-16, B-SA-17, B-SA-18, B-SA-20, B-SA-21, B-SA-23, B-SA-24, B-SA-25 and sanober-96, "moderately susceptible" (MS) included three genotypes that were B-SA-22, B-SA-4 and B-SA-26 and "Susceptible" (S) in terms of disease resistance were included five genotypes that were B-SA-3, B-SA-8, B-SA-9, B-SA-26, and local barley genotype Ajj with having AUDPC valves ranged from 1-100, 101-200, 201-300 and above 300 respectively. Assessment of wheat and barley verities against *BYDV* resistance across different seasons was carried out. The fluctuation of *BYDV* symptoms was observed between years and genotypes and was shown to be greatly influenced by ecological factors (Comeau and Jedlinski, 1990; Eberhart and Russel, 1966). According to Bashir *et al.*, (1997) in Khyber Pakhtunkhwa five different *B/CYD* serotypes (*BYD-PAV, BYD-RMV, CYD-RPV, BYD-MAV*, and *BYD*-SGV) from dissimilar areas of Peshawar valley have been detected in wheat crop. Similarly Aheer *et al.*, (2006) reported the detection of five different species damaging wheat crop *viz. Rhopalosiphum maidis* (Fitch), *Sitobion avenae* (Fab),*Schizaphis graminus* (Roudoni), *Rhopalosiphum padi* (L.) and *Rhopalosiphum rufiabdominalis* (Sasaki).

Conclusion

It is concluded from our findings that the incidence and severity of BYDV were found high in 2017 as compared to the studied year 2016. Furthermore, the environmental factors including temperature and humidity were found to have a key role in BYDV vector attraction and spread on the host plant.

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