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Research Article

A Study on Variability in Resistance to Barley Yellow Dwarf Virus (BYDV-PAV) among Ethiopian Barley (*Hordeum vulgare* L.) Landraces

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ABSTRACT

Ethiopian barley landraces are recognized as an important genetic resource with which to search for tolerance to biotic stresses. Five hundred eighty five barley (*Hordeum vulgare* L.) landraces collected from 13 barley producing zones of Ethiopia were evaluated for resistance to BYDV serotype PAV under field conditions at Sinana and Goba during 2018 and 2019 cropping seasons. The trial was laid out in an augmented design consisting of six blocks. Data on disease incidence, severity and some agronomic traits were recorded. The result of analysis showed highly significant variations among landraces for disease incidence, severity and agronomic traits. The BYDV incidence and severity varied from 0.0 to 45.3% and 12.0 to 58.0%, respectively. Similarly, the variations among landraces in terms of regions of origin, altitude classes and kernel row number were significant. Landraces originated from Arsi, Gojam, Bale and Gonder had significantly lower disease incidence and severity levels than others. Furthermore, landraces collected from altitude class IV (above 2500m) and those with 6-row and irregular kernel type had lower disease severity. Segregation in infection was observed in some landraces due to a reduction of disease severity and 68 landraces showing lowest disease severity were selected. The resistant landraces identified in the present investigation can be utilized as good resource for barley improvement program targeting BYDV, which subsequently will help to incorporate the resistant genes into several elite backgrounds of barley.

Keywords: Barley; BYDV-PAV; Landraces; Resistance; Variability

INTRODUCTION

In Ethiopia, barley (*Hordeum vulgare* L) is the fourth cereal, cultivated in all the regions of the country. The country is considered as a center of diversity and landraces form the major genetic resources of cultivated barley in the country [1,2]. Ethiopian barley landraces had several important traits, such as disease resistance to Barley yellow dwarf virus-PAV (BYDV-PAV), powdery mildew (*Erysiphe graminis*), barley leaf rust (*Puccinia hordei* Otth.), net blotch (*Pyrenophora teres Drechsler*), viruses and barley scald (*Rhynchosporium secalis*) [3,4]. Further, according to the study report by Rasmusson and Schaller [5], Niks et al. [6] and Edwards et al. [7], Ethiopian barley landraces are the only sources for resistance to BYDVs and genetic resistance is the best practical solution to alleviate effects of the diseases. Earlier studies reported that the BYDV resistant gene Yd2 [5] and Yd3 [6] have been identified from Ethiopian barley landraces and extensively used in breeding programs throughout the world to develop BYDV resistance.

The adaptability of landraces to a wide range of environmental conditions is recognized as an important genetic resource with which to search for tolerance to biotic and abiotic stresses [8]. So far landrace potential has not been fully realized in modern breeding [9]. One of the main reasons for the limited use of landraces to introduce new genetic variation into breeding programs was linkage drag [10]. However, the advancement molecular markers platforms, proved to be a solution to overcome this problem [11] and the use of these platforms is becoming routine in crop breeding programs [12]. Recently, it is for this reason that across the world efforts are started to utilize landraces and wild species for new and efficient sources of resistance to BYDV to combine them with already used modern cultivars in order to increase the resistance durability of the crop.

In Ethiopia, currently, there are more than 15,000 barley accessions in the Ethiopian Biodiversity Institute (EBI) gene bank. However, majority of the collected materials are not yet characterized in detail for their morphological diversity and very little research has been documented to recommend specific barley accessions as effective resistance sources for BYDV [13]. Therefore, this study was aimed to assess the existing phenotypic and genetic variability among barley landraces in response to BYDV-PAV resistance by using large

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scale testing materials in order to expand varietal choice options for the researcher in developing barley cultivars with better agronomic performances and resistant to BYDV-PAV disease stresses for current agricultural system resilience.

MATERIALS AND METHODS

The field experiments were conducted during 2018 and 2019 main cropping seasons at Sinana Agricultural Research Center (SARC) and Goba research sites, south-east, Ethiopia. The two sites were selected due to their BYDV-PAV hotspot where it occurs regularly in the region and representing high altitude of barley production. Both areas have bimodal rainfall pattern.

Five hundred eighty five barley landraces collected from 13 barley cultivating zones along with 10 checks were evaluated over a period of two years (2018 and 2019 main cropping seasons) in an augmented design consisting of six blocks. The plot size used was single-row with 1.75 m length and 0.2 m between rows. Seeds were planted manually with a seeding rate of 100 kg/ha. Agronomic practices like fertilizer application and weed control were applied to both experiments. Data on 10 quantitative characters (Table 1) as barley descriptor [14] and disease responses were scored.

Disease assessment

Symptomatic reactions (VSS-Visual Symptom Score) to the BYDV, serotypes PAV which is common in the area, among five BYDV serotypes (PAV, MAV, RPV, RMV, SGV), incidence (number of infected tillers/plot) and severity (percentage of foliage with symptoms) was carried out according to 0-9 scale as described by Schaller and Qualset [15] and Singh et al. [16] in early stage due to the expression of BYD symptoms and peak activity period of its viruliferous aphid vectors (*Rhopalosiphum padi* L.) in the area [13] at booting stage (41-49 Zadoks scale) [17]. Presence or absence of leaf tip necrosis (LTN) on flag leaf of each landrace was recorded over two years (2018-2019) as mentioned by Shah et al. [18].

RESULTS

Variation levels among barley landraces

The disease responses and agro-morphological characters recorded showed a wide range of variations (Table 2). From descriptive statistics, lowest BYDV disease incidence (number of infected tillers/plot) was 0.0% and the highest value was 45.3%. The estimated mean value was -15.4%. On the other hand, the computed BYDV severity (percentage of foliage with symptoms) ranged from 12.0% to 58.0% with an average value of 27.0 (Table 2). Days to heading and maturity ranged from 53.1-88.0 and 94.0-136.0 days, respectively. The range of grain yield plant-1 varied from 857.1 to 6,460 kg ha^{-1} with average yield of 1740.0 kg ha^{-1}. The difference among the 585 landraces in terms of assessed quantitative characters was highly significant.

As shown in Table 3, the lowest barley BYDV incidences and severity were recorded on landraces collected from Arsi, Gojam, Bale and Gonder zones. In contrast, incidence and severity were the highest for landraces from Gamo Gofa, Hadiya, Hararghe, Jimma and Tigray zones. In this study, out of 585 landraces tested, 63.2% (370 accessions) have highly significantly lower severity than the susceptible checks “Guta and HB-42”.

Variability in terms of altitude classes and kernel row number

The altitudes of origin were classified into four classes, viz; altitude class I (<1500m), altitude class II (1501-2000m), altitude class III (2001-2500) and altitude class IV (>2500m). Kernel row number also classified to three kernel types, viz; 2-rowed, 6-rowed and irregular barley types. Landraces collected from the four altitude classes were significantly different in disease response and all agronomic characters over two years and two locations. The computed mean squares from combined ANOVA for BYDV response and agro-morphological characters in terms of altitude classes and kernel row types are shown in Table 4. Highly significantly ($P<0.01$) and lower estimated mean values of BYDV incidence and severity were recorded for landrace collected from altitude class IV (above 2500 m) as compared to landraces originated from other remaining altitude classes. Landraces from this altitude class had long heading and maturity periods. Regarding agro-morphological characters, significantly ($P<0.01$) higher values were recorded for fertile tillers plant-1, seeds spike-1, 1000-seed weight and grain yield plant-1 for this altitude range than for the other three altitude classes. In terms

Table 1: Quantitative characters recorded (IPGRI, 1994).

Characters	Description
Days to heading	Recorded as the number of days from planting to the date on which approximately 75% tillers had produced spikes.
Days to Maturity	Recorded as the number of days from planting up to the time when 90% of the plants in a row had reached physiological maturity (the stage when color of plant changed from green to golden yellow and its tillers could break easily with hands).
Thousand-seed weight	Weight in grams of 1000 seeds randomly taken from each experimental plot (at 12.5% moisture content).
Grain yield	The grain yield of each experimental unit after moisture content was adjusted to 12.5% and expressed in grams.
Spike length	Recorded as length of the spike in cm on the tallest culm (excluding awn).
Number of seeds per main spike	Recorded as the total number of kernels in the main spike from each plant.
Plant height	Height in centimeter from the soil surface to the tip of the spike (awn excluded) of the tallest culm.
Number of fertile tillers per plant	Recorded as the total number of seed-bearing (fertile tillers) for each plant.
Peduncle length	Measured as the length of peduncle in centimeters from the last node to the base of spike, as the average of 10 randomly taken plants from each accession.
Flag leaf length	Length in centimeters from the leaf tip to collar of flag leaf.

Table 2: Descriptive statistics and broad sense entry-mean heritability for BYDV tolerance in 585 barley landraces.

Characters	Min.	Max.	Mean	Standard deviation	Variance	CV (%)	H^2
Number of infected tillers (Incidence)	0.0	45.3	15.4	13.2	23.1	18.8	65.0
% of foliage with symptom (Severity)	12.0	58.0	27.0	6.5	51.7	21.5	23.5
Leaf tip necrosis	1.3	4.6	1.7	3.9	11.3	7.8	43.9
Degree of attack	0.0	31.0	44.0	4.9	4.3	9.1	47.8
Days to heading	53.0	88.0	67.9	6.4	41.4	6.16	73.51
Days to maturity	94.0	136.0	112.6	9.3	86.6	6.17	59.65
Flag leaf length	4.8	26.8	15.5	2.9	8.4	14.27	49.14
Plant height	63.2	122.8	96.6	9.1	82.3	6.92	82.83
Peduncle length	12.6	48.5	29.6	6.7	44.4	10.26	28.49
Spike length	4.4	12.7	8.3	1.4	1.9	9.66	81.11
Grain yield	857.1	6460.0	1740.0	40.2	116.0	47.36	72.16
Fertile tillers per plant	0.6	12.5	3.2	1.5	2.2	20.56	88.91
Seeds per spike	12.3	76.2	37.4	13.3	177.3	22.58	81.80
Thousand seed weight	8.2	53.2	31.0	7.6	58.3	14.33	82.53

Table 3: Variations in quantitative traits and BYDV-PAV intensity of the 585 barley landraces across the experimental sites and over two years.

Characters	Zones of origin												
	Arsi	Bale	G/Gofa	Gojam	Gonder	Hadiya	Hararghe	Jimma	Shewa	Sidama	Tigray	Wellega	Wello
NIT	1.3**	1.3**	1.6**	1.3*	1.3**	1.4**	1.6**	1.6**	1.5*	1.6*	1.7**	1.5*	1.5*
FWS	33.3**	34.0**	36.9**	34.3**	34.7**	38.9**	39.7**	38.4	37.3**	37.6**	35.6**	35.7**	39.0**
LTN	0.6*	0.7*	0.7*	0.6*	0.7*	0.7*	0.7*	0.7*	0.7*	0.6*	0.7*	0.6*	0.7*
DA	55.8**	49.8**	40.7**	74.4**	55.3**	68.7**	59.3**	96.4**	75.4**	21.4**	25.3**	79.2**	38.8**
DH	35.6**	19.5**	11.9**	93.6**	122.3**	26.7*	21.0*	14.0*	61.5*	66.5**	36.3**	40.8**	46.3**
DM	76.0**	73.4**	57.8**	135.2**	159.0**	73.8ns	80.8*	89.7ns	97.9**	131.6**	107.0**	117.5**	70.2**
FLL	9.79**	13.1**	13.9**	14.0**	13.0**	12.7**	7.3*	11.1**	13.6**	11.3**	10.4*	8.5*	12.3*
PH	72.2**	80.2**	79.2*	136.9**	53.9**	111.9**	50.1*	64.6**	72.2**	84.2**	99.5**	74.9**	32.0**
PL	9.8**	13.1**	13.9**	14.0**	13.0**	12.7**	7.3**	11.1**	13.6**	21.5**	10.4**	8.5**	15.8**
SL	1.9**	1.3**	2.2**	0.2**	1.0**	4.3**	1.4**	2.6**	2.1**	2.3**	1.2**	1.2**	2.7**
GY	272.9**	926.6**	470.2**	253.0**	207.0**	181.1**	586.4**	212.2*	390.1**	139.2**	189.9**	186.5**	190.5**
FTPP	2.6**	2.5**	2.6*	6.4**	4.8**	4.3**	1.6**	1.9**	5.1**	2.8**	5.3**	2.7**	5.0**
SPS	224.6**	145.7*	216.7*	104.3*	208.2*	303.4**	183.3**	365.3**	209.5**	201.9*	98.7**	198.3**	235.5**
TSW	49.0**	35.4**	74.9**	67.1**	59.1**	91.2**	27.4**	25.4**	27.3**	33.4**	36.0**	69.6**	53.0**

NIT = Number of infected tillers (Incidence) , FWS = % of foliage with symptom (Severity), LTN = Leaf tip necrosis, DA = Degree of attack, DH = Days to 50% heading, DM = Days to maturity (days), FLL = Flag leaf length (cm), GY = Grain yield per plant (g), FTPP = Number of fertile tiller per plant (number), SPS = Number of seeds per spike (number), PH = Plant height (cm), PL = Peduncle length (cm), SL = Spike length (cm), TSW: Thousand seed weight (g).

Table 4: Variability of barley landrace lines originated from three ranges of altitudes and three ear-types to *Puccinia hordei* and other characters across two locations and 2 years (2018 and 2019).

Characters	Altitude					Kernel row number			
	<1500	1501-2000	2001-2500	>2500	Mean	2-rowed	6-rowed	Irregular	Mean
Number of infected tillers (Incidence)	1.6a	1.5b	1.5b	1.4c	**1.5**	1.5a	1.4b	1.4b	**1.5**
% of foliage with symptom (Severity)	35.2c	37.1b	37.6a	34.1d	**36.7**	36.3b	37.1a	34.3c	**36.7**
Leaf tip necrosis	0.9a	0.7b	0.7b	0.6c	**0.7**	0.7a	0.5b	0.7a	**0.7**
Degree of attack	53.4a	34.7b	27.1c	25.2d	**37.1**	57.6a	39.0b	28.7c	**39.7**
Days to heading	51.2c	64.0b	67.2a	67.8a	**66.5**	59.4c	67.3a	62.0b	**64.7**
Days to maturity	97.3c	109.7b	112.3ab	115.0a	**103.7**	91.2b	108.7a	107.4a	**104.4**
Flag leaf length	12.2c	14.9b	15.8b	19.8a	**16.1**	9.3c	12.8a	11.7ab	**13.7**
Plant height	97.2c	98.1b	98.2b	101.9a	**102.3**	94.3b	95.7ab	97.3a	**96.5**
Peduncle length	29.4b	29.1c	29.7ab	30.0a	**29.7**	29.2b	29.9a	29.7ab	**29.6**
Spike length	8.3b	8.4a	8.3b	8.3b	**8.3**	8.9a	7.9c	8.3b	**8.4**

Grain yield	63.9cd	65.7c	78.3a	70.4b	**69.4**	61.4c	82.1a	69.5b	**67.4**
Number of fertile tillers per plant	3.4c	3.5b	3.8a	3.6ab	**3.7**	3.2c	3.9a	3.4b	**3.6**
Number of seeds per spike	39.7bc	37.8b	48.4a	39.1c	**38.3**	35.1b	39.7a	36.1b	**37.7**
Thousand seed weight	29.6c	31.3b	34.3a	33.8a	**30.7**	31.7b	34.9a	31.6b	**30.7**

Mean values with same letter are non-significant and with different letters are highly significant at P = 0.01 (LSD$_{01}$)

Table 5: Distribution of 585 barley landraces collected from 13 zones, four altitude classes and kernel row number over severity (%) classes of BYDV-PAV with a class interval of 10 units.

Categories		Severity classes (%)										Total
		0-10	Nov-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100	
Kernel row number	2-rowed	5	11	59	70	48	26	7	1	0	0	227
	Irregular	4	3	5	16	7	4	2	0	0	0	41
	6-rowed	6	21	71	99	65	34	14	7	0	0	317
Altitude classes	<1500	0	0	1	5	4	1	2	1	0	0	14
	1501-2000	6	5	40	44	36	15	8	1	0	0	155
	2001-2500	6	15	54	91	50	33	10	6	0	0	265
	>2500	3	14	36	47	30	15	5	1	0	0	151
Zone	Arsi	2	6	15	33	13	12	2	0	0	0	83
	Bale	0	0	15	12	11	4	1	3	0	0	46
	Gamo Gofa	2	2	7	11	7	5	2	0	0	0	36
	Gojam	3	3	12	23	10	6	2	0	0	0	59
	Gonder	1	3	10	16	12	4	1	0	0	0	47
	Hadiya	0	1	3	8	7	1	1	0	0	0	21
	Hararghe	1	2	11	21	7	11	3	1	0	0	57
	Jimma	0	0	6	5	7	1	1	0	0	0	20
	Shewa	1	8	18	12	22	9	4	1	0	0	75
	Sidama	1	2	4	5	4	0	0	1	0	0	17
	Tigray	2	2	17	25	11	4	2	1	0	0	64
	Wellega	2	4	9	7	3	2	2	0	0	0	29
	Wello	0	2	8	7	6	5	2	1	0	0	31

of kernel row number, higher and significant (P<0.01) differences were recorded on 2-row barley types for both disease incidence and severity score and all agro-morphological characters except 1000-seed weight. Irregular and 2- rowed barley types had the lowest scores for disease and agro-morphologic characters (Table 4).

Distribution of landraces over BYDV severity classes

Landraces were grouped based on BYDV disease severity (percent foliage with symptoms) with an interval of 10 units in terms of kernel row number, altitude classes and regions of origin, as shown in Table 5. The distribution in terms of zones of origin varied from five severity classes for landraces from Jimma to eight classes for landraces from Hararghe, Shewa and Tigray. Other remaining landraces from Bale, Hadiya and Sidama zones and Arsi, G/Gofa, Gojam, Gonder, Wellega and Wello were distributed into six and seven severity classes, respectively. Further, in terms of altitude classes, landraces from three altitude classes, Viz, class II, III and IV distributed over eight severity classes; whereas those from altitude class I distributed into five classes. According to kernel row number, the landraces were distributed into 8, 8 and 7 severity classes for 2-rowed, 6-rowed and irregular barley types, respectively.

DISCUSSION

The Ethiopian highlands, where farmers cultivate landraces [19,20] are known to be good sources of variation. These barley landraces are found in diverse agro-ecologies, developed over several years and are considered as genetically adapted to the existing BYDV pathogen populations and still give fairly good and stable yields. This long time adaptation and their ability to give fair and stable yields despite of such disease stresses suggest that landraces must carry some kind of durable resistance. Further, the globally identified two BYDV-PAV resistant major genes (Yd2 and Yd3), for instance, exclusively were from the Ethiopian barley landraces [5,6] and have since been used to improve resistance of spring and winter barley cultivars and breeding lines [21]. However, this potential of the landraces had not yet been exploited in barley improvement programs in the country.

In present investigation, 585 landraces collected from 13 barley producing zones of Ethiopia were evaluated for their resistance to BYDV; serotype PAV, which is widely found in experimental fields among five serotypes (PAV, MAV, RPV, RMV and SGV). This serotype had significant effect with visual field incidences reaching as high as 80-100% and most identified type [13]. In 2018 and 2019 cropping seasons, the weather conditions (both temperature and rainfall) were conducive for the occurrence of cereal-aphid, insect transmitting BYD and as a result, higher incidence (45.3%) and severity, (58.0) were recorded. From the analysis, it was also observed that the landraces grown responded differentially to BYD epidemics. They were significantly different in disease response (both in terms of incidence and severity) and agro-morphological characters recorded. About 63.2% of the landraces have significantly lower disease record and relatively higher grain yields plant-1 compared to susceptible checks, Guta and HB-42. On the other hand, 184 (31.5%) landraces had closely similar BYD disease severity as to the susceptible lines and 31 landraces appeared significantly more susceptible than the checks. In the study undertaken by Bekele et al. [13] to screen landraces for multiple disease resistance, higher proportions of barley landraces had significantly lower disease incidence and severity. Schaller and Qualset [15] found that about 21% of barley landraces from Ethiopia were resistant to BYDV.

The altitude of origin classified into four classes and the infection responses of barley landraces to BYDV varied based on the altitude of origin. Accordingly, out of 151 landraces collected from altitude class IV (>2500 m), 35.1% had significantly lower BYDV severity than susceptible checks. From altitude class II (1501–2000 m.a.s.l.) and III (2001-2500 m.a.s.l.), 35.5% of 155 and 28.3% of 265 landraces had lower disease severity than the susceptible checks, respectively. Further, 68 out of 416, least infected landraces were collected from the highest altitude area of the country. Seven percent of the landraces collected from altitude class I (<1500 m.a.s.l.) showed lower disease severity. However, from research results reported by Qualset [22] and Bekele et al. [13], Ethiopian barley landraces collected from high elevations had a much higher frequency of BYD resistance than barleys from the lower areas. The observed increase in resistance to diseases at higher elevation is best explained as an adaptation of the landraces to the fast increasing disease pressure with increasing altitude. Furthermore, the increase in resistance of landraces with increase in elevation to viral diseases observed in this study confirmed the work of Qualset [22] and Bekele et al. [13] who reported earlier that the Ethiopian landraces originated from highland areas are good sources for resistances to BYDV.

In terms of kernel row number, 33.0%, 30.9% and 29.3% the two-rowed, six-rowed and irregular barley types, respectively had significantly lower disease severity (<30.0%) than susceptible checks. Out of barley landraces showing reduced epidemic levels, the least infected sixteen, seven and twenty seven were from 2-rowed, 6-rowed and irregular barley types, respectively.

CONCLUSION

A study on some agronomic traits, those resistant barley landrace had long maturity period, higher plant heights, fertile tillers plant-1, 1000-seed weight and grain yields plants-1. This showed the occurrence of barley BYDV has negative correlation with yield and yield components indicating taller barley landraces had lower epidemic build-up of barley BYDV and giving more yields when the disease was less severe. From among the 585 landraces evaluated, 370 landraces had highly significantly lower BYDV severities than susceptible checks “Guta and HB-42” under field conditions and sixty-eight landraces showed significantly lower disease severity than the local check (Aruso). Therefore, those barley landraces with lower disease severity could be used in barley improvement programs as resistance sources to BYDV and future evaluation of more barley landraces from the gene bank of Ethiopian Biodiversity Institute and expand germplasm choice options for the researcher in getting valuable disease resistant genes. Also the novelty of these sources of resistance must be investigated further and will be worthwhile to conduct mapping studies to find out markers closely linked to resistance genes and allow their efficient selection in barley breeding and to determine their association with other known resistance genes.

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