Ethiopian barley landraces: useful resistant sources to manage *Barley yellow dwarf* and other foliar diseases constraining productivity

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Abstract One hundred sixty-five advanced lines were subjected to marker-assisted selection using Cleaved amplified polymorphic sequence-polymerase chain reaction (CAPS-PCR) primer against *Barley yellow dwarf* (BYD) resistant *Yd2* gene. In addition, phenotypic evaluation was employed to screen the advanced lines for multiple disease resistance. Effect of host growth rate and incubation period on BYD development was also assessed. Results of marker PCR showed that *Yd2* gene was detected in 96 (58.2%) out of 165 accessions tested. Data on relationships of days to heading (DtH) and BYD symptoms development revealed that barley accessions having shorter DtH (66 days), BYD severity appeared low, and vice versa on accessions having longer DtH (79–83 days). Screening for multiple disease resistance gave interesting results. Among 11 accessions

(with *Yd2*) selected as the best for multiple diseases, 6 accessions [Chelia local, 4304–2(2), 4818(1.3), 1822–1(1), 1831–2-2(2), and 4915–1(1.2)] were highly resistant to leaf rust and net blotch, while other five lines had moderately resistant reaction. Study on the relationship of host resistance and incubation period showed that BYDV was detectable 5 days earlier in susceptible than in resistant lines. This can be used as an alternative method in preliminary screening of large number of genotypes against BYDV. From this study, it was evident that the identified resistant genotypes are a good resource for barley improvement program targeting BYDV and other important foliar pathogens. Barley breeders are encouraged to take advantage of this opportunity in producing multi-disease resistant barley genotypes in an agronomically enhanced genetic background in the country and beyond.

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Introduction

Barley (*Hordeum vulgare* L.) is among the most economically important cereals grown in Ethiopia. In terms of area coverage and total production, barley ranks 5th next to tef, maize, sorghum and wheat (CSA 2016). It covered over one million ha with a total production of 2.0 million tones, and productivity of 1.81 t/ha. Biotic stress such as diseases take the lion share accounting for such low productivity. Ethiopia is an important primary and secondary gene

center for many field crop species, including barley with wide genetic diversity (Adugna 2011; Harlan 1969; Vavilov 1951). Several traits, such as disease resistance to *Barley yellow dwarf virus-PAV* (BYDV-PAV), powdery mildew, leaf rust, loose smut, and *Barley stripe mosaic virus* (BSMV), and high lysine content were identified from barley germplasm originated from Ethiopia (Abebe 2006). The availability of resistance traits for various economically important diseases is an opportunity to be exploited to develop multiple disease resistance.

Breeding new varieties through conventional methods take up to 12 years and even then, the release of an improved variety cannot be guaranteed (Korzun 2003). The advent and applications of molecular marker technologies worldwide, however, is changing the scene significantly by speeding up the classical crop improvement program by assessing and/or introgression of rich gene pool that exists in the cultivated crops and their wild relatives. Molecular markers are of paramount importance in serving as chromosomal landmarks to trace the presence of a specific genomic region related to a useful trait during the breeding process. Consequently, they are helpful to accumulate multiple genes for resistance to specific pathogens within the same cultivar (gene pyramiding). Besides mapping and tagging genes, molecular markers have made it also possible to map and characterize the polygenes underlying quantitative traits in natural populations (QTL mapping). Molecular diagnostic methods based on polymerase chain reaction (PCR) technology, such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and simple sequence repeat microsatellite (SSR) emerged in the 1980s and early 1990, and their use to enhance plant breeding efforts has been described by many investigators (Dudley 1993; Paterson et al. 1991; Stuber et al. 1999).

Located on chromosome 3H (Schaller et al. 1964), the BYDV resistance gene (*Yd2*) was first identified by Rasmusson and Schaller (1959) in barleys originated from Ethiopia. Later, Collins et al. (1996) has accurately mapped and demonstrated that *Yd2* gene co-segregated with RFLP markers Xwg889 and XY1p on the long arm of chromosome 3, about 0.5 cM from the centromere. Sequence data further showed that the Ylp alleles differ by a single nucleotide in barley with and without *Yd2*. Such close linkage suggested to provide an opportunity to develop a strong marker for *Yd2* selection. Paltridge et al. (1998) and Jefferies et al. (2003) were also developed a co-dominant PCR-based marker designated

YLM. More recently, the other resistant gene '*Ryd3*' was identified from barley and linked to the SSR markers HVM22, HVM14, HVM65, HVM74, Bmac0018 and Bmac0009 (Niks et al. 2004). *Yd3* located on a different chromosome (chromosome 6H) (Spaner et al., 1998; Ramsay et al., 2000) than *Ryd2*, and their location on a different chromosome suggested as an advantage to easily combine both genes in one cultivar to attain a higher level of BYD resistance than either alone. These resistant genes are currently widely used all over the world in barley improvement programs. Whilst both genes were identified from the Ethiopian barley landrace collections, they were not incorporated into elite barley cultivars.

Barley is challenged by the presence of complex of more than one disease that commonly occur in farmers' fields at the same time. Leaf rust (caused by *Puccinia hordei* Otth.) and net blotch (caused by *Pyrenophora teres* Drechsler) are, among others, the two major fungal diseases constraining barley production in major growing areas in Ethiopia (Ayele et al. 2008). Among cereal viruses, BYDV-PAV is causing significant impact on highland grown barley in Ethiopia (Agranovsky 1986; Bekele 1998; Bekele et al. 2001, 2003). The level of damage caused by these diseases necessitated development of efficient control measures. Since chemical application is not an economically or environmentally sound solution, the development of varieties with durable resistance is a priority for breeding programs. The aim of this investigation was: primarily, to screen barley accessions/landraces resistant to BYDV, using *Yd2* linked marker (YLP) (Ford et al. 1998), and phenotypic evaluation for leaf rust and net blotch; secondly, to determine whether differences exist between lines with and without *Yd2* gene on virus incubation period and/or movement; and thirdly, to understand if there is any association between plant growth rate [days to heading (Dth)] and BYD severity.

Materials and methods

Evaluation of barley accessions to BYDV-PAV, leaf rust and net blotch

Plant materials and DNA extraction Plant materials used in this study was earlier selected and advanced from around 1400 barley landrace collections and released cultivars obtained from Ethiopian Biodiversity

Institute (EBI) and Barley Improvement Department of Holetta Agricultural Research Centre (HARC) (PPRC 2012). The selected materials were further purified for “true to type” based on barley descriptors using morphological features (IPGRI 1994). A total of 165 genotypes, of which 143 barley landrace collections identified earlier at Plant Protection Research Center (PPRC) as promising lines against BYDV-PAV under field conditions and 22 released and elite candidates were evaluated using PCR marker (*Yd2*-linked marker) to determine whether the known BYDV-PAV resistance gene is involved in the field observed resistance reactions. Each genotype was planted in un-replicated, two row plots of $0.6 \text{ m} \times 1 \text{ m} = 0.6 \text{ m}^2$, with spacing of 0.3 m and 0.5 m, between rows and plots, respectively. About 1 g barley plants were sampled at 1–2 leaf growth stages (11–12 Zadoks scale) (Zadoks et al. 1974) for DNA extraction. Total plant DNA was extracted using cetyl trimethyl ammonium bromide (CTAB) buffer as essentially described by Doyle and Doyle (1990) with the modification that samples were ground in liquid nitrogen using tissue layer. All other plants of each genotypes were allowed to grow until maturity to monitor development of BYD symptoms and other foliar diseases. To guarantee BYD development, cultivars “Gold (CI 1145)” and “HB-42” were planted at a uniform interval as a source of infection row one weak ahead of test genotypes. In addition, to supplement natural infection, viruliferous aphid vectors (*Rhopalosiphum padi* L.) reared at PPRC greenhouse were uniformly distributed on test plants to promote infection. Superimposed on the same barley materials, host response to leaf rust and net blotch were phenotypically evaluated. Data on heading date, BYD symptoms severity (0–9 scale) (Schaller and Qualset, 1980) at booting stage (41–49 Zadoks scale) (Zadoks et al. 1974), and leaf rust (*Puccinia hordei* Otth.) and net blotch (*Pyrenophora teres* Drechs.) severity and plant response to infection were recorded twice at heading and flowering using modified Cobbs Scale (Peterson et al. 1948) (Table 1).

Polymerase chain reaction (PCR) analysis for *Yd2* gene PCR amplification of *Yd2* gene was performed in 15 µl reaction volume containing 3 µl Buffer, 8.85 µl SdH_2O , 10 pmole each primer (0.5 µl each), 0.15 µl Taq polymerase and 2 µl genomic DNA. PCR was run on a standard thermocycler with reaction conditions of 94 °C for 5 min followed by 35 cycles of 94 °C for 30 s, 53 °C for 1 min and

72 °C for 2 min, and a final extension step of 72 °C for 5 min. The CAPS-PCR primers (Ford et al. 1998) used for the amplification of the *Yd2* gene were Y1p-MF (AATACAGGAATCTGTTGAAAGAA) and Y1p-MR (TCATCATGGCTCGGAGAAGGTGG). PCR products (10 µl/well, each) were separated by 2.5% agarose gel electrophoresis in 1 x TAE (Tris-Acetate-EDTA) buffer stained with 5 µl Redsafe™ nucleic acid staining solution (20,000x) (iNtRON Biotechnology) and visualized using UV light transillumination. For reference and comparison of PCR products, 100 bp DNA size marker (iNtRON Biotechnology) was used along with the samples at 4 µl/well.

Relationships of virus incubation period and host plant resistance

Plant materials and virus inoculum preparation Sixty-seven barley accessions (Table 2), randomly selected from 165 field selected advanced barleys, were evaluated in the greenhouse to determine association of BYDV-PAV incubation period or movement and host plant resistance. Based on the PCR results (above), both susceptible (*-Yd2*) and resistant (*+Yd2*) genotypes were included. All accessions were planted in pots in greenhouse at ambient room temperature. Culture of local BYDV-PAV isolate earlier identified at PPRC were used in this study. Similarly, for virus acquisition and inoculation, a local colony of *R. padi* was reared in greenhouse at ambient room temperature (~ 22 °C). Test plants were grown in pots of 23 cm × 20 cm, with each pot planted to six seeds for each treatment and maintained in the growth house with day temperature of 20–22 °C. A 48 h acquisition and inoculation access periods adopted, and 10 viruliferous aphids per plant were used for inoculation feeding. All plants receiving different treatments were inoculated equally at 2–3 leaf stages. Six treatments were tested, namely 2, 5, 10, 15, 20 and 25 days after inoculation (DAI).

Laboratory analysis using tissue blot immunoassay (TBIA) Plants of each treatment were tested by tissue blot immunoassay (TBIA) test as essentially described by Makkouk and Comeau (1994). Stems of test plants from each treatment were cut using new razor blades and blotted on nitrocellulose membrane (NCM) and analyzed by the TBIA. A

Table 1 Cleaved amplified polymorphic sequence-polymerase chain reaction (CAPS-PCR) amplification of *Yd2* gene and reaction of barley genotypes to *Barley yellow dwarf virus* (BYD), leaf rust and net blotch at Ambo, Ethiopia, 2015/2016 main cropping seasons

No.	Accession	Days to heading	<i>Yd2</i> gene	BYD symptoms severity (0–9)	Net blotch severity (%)	Leaf rust severity (%) and response
1	1785-1-1	66	+	0	25	7MR
2	3324-1-1(2)	66	+	3	5	30S
3	3412(2)	66	–	2	65	45S
4	3492–2(2)	66	+	0	65	35S
5	3675–1(4)	66	+	0	25	25S
6	4390–1(2.1)	66	–	0	30	50S
7	4390–2(1)	66	–	0	15	85S
8	Shenen local(4)	66	+	0	8	20S
9	4199(2)	67	–	0	55	50S
10	1831–2-2(1)	69	+	1	10	5MR
11	1836(1)	69	+	0	5	20MS
12	1836(2)	69	–	1	5	20MS
13	1838–1	69	–	0	10	30MS
14	3167–2	69	–	2	10	40S
15	3324-1-1(1)	69	–	0	3	40S
16	3326-1-1	69	+	0	45	25S
17	3412(1)	69	+	0	15	20S
18	3492–2(1)	69	+	0	45	40S
19	3492–2(1.2)	69	–	0	65	35S
20	3492–2(3)	69	–	2	30	40S
21	3492–2(3.2)	69	–	0	65	55S
22	3675–1(1)	69	–	0	65	20MS
23	3675–1(2)	69	–	5	65	20S
24	3675–1(3)	69	–	2	35	60S
25	3988–1(1)	69	–	0	15	20MS
26	4075-2-3(1)	69	–	0	20	30S
27	4075-2-3(3)	69	–	0	10	45S
28	4199(1)	69	–	2	30	65S
29	4199(3)	69	–	0	20	50S
30	4228-1-2(1)	69	–	0	15	45S
31	4228-1-2(2)	69	+	1	25	35S
32	4228-1-2(3)	69	+	0	25	45S
33	4228-1-2(4)	69	–	0	30	50S
34	4228–2(1)	69	+	0	20	50S
35	4228–2(2)	69	+	2	15	65S
36	4238–2(1)	69	–	2	30	60S
37	4243-1-1	69	+	0	3	25S
38	4253(1)	69	+	0	40	30S
39	4253(2)	69	+	1	15	45S
40	4264(2)	69	–	0	15	70S
41	4290–2(2)	69	–	0	25	60S
42	4291–1(1.2)	69	+	0	35	40S
43	4300–1(2)	69	–	0	25	40S

Table 1 (continued)

No.	Accession	Days to heading	<i>Yd2</i> gene	BYD symptoms severity (0–9)	Net blotch severity (%)	Leaf rust severity (%) and response
44	4300–1(3)	69	+	0	15	85S
45	4300–1(4)	69	+	0	15	50S
46	4312	69	+	1	20	50S
47	4304–2(1)	69	+	0	15	45S
48	4390–1(1.1)	69	–	4	20	35S
49	4390–1(1.2)	69	–	1	10	35S
50	4390–1(1.3)	69	+	2	15	55S
51	4418–2–2(1)	69	–	0	15	30S
52	4756(1)	69	–	2	30	50S
53	4756(2)	69	+	0	15	45S
54	4756(4)	69	+	0	15	90S
55	4765–2	69	+	0	25	40S
56	4767–1–1	69	+	2	15	30S
57	4806–1(1)	69	+	1	15	20S
58	4841–2(1.3)	69	+	0	20	5MR
59	4883	69	+	0	20	25S
60	4915–1(1.1)	69	+	0	15	80S
61	64,016–1–2	69	+	1	8	45S
62	64,105(1.1)	69	+	4	15	85S
63	64,106–2–1	69	+	3	2	75S
64	64,178(1.1)	69	–	5	5	30S
65	64,223(2)	69	+	0	15	25S
66	64,223(3)	69	+	3	5	30S
67	64,226–1	69	+	4	7	40S
68	HB-42(11)	69	+	4	10	30S
69	IBON 170/03	69	+	2	1	5R
70	Chelia Local (1)	69	+	3	<1	<1R
71	4238–2(2)	70	–	2	15	60S
72	4239–2–1	70	–	2	5	35MS
73	4239–2–2(1)	70	–	0	5	25S
74	4385	70	–	2	30	30S
75	4264(1)	71	+	0	15	65S
76	4290–2(1)	71	+	0	65	85S
77	4291–1(2.1)	71	+	0	40	45S
78	4291–1(2.2)	71	+	0	50	30S
79	4291–1(3.1)	71	–	0	50	45S
80	4300–1(1)	71	+	0	15	60S
81	4304–2(3)	71	+	1	15	45S
82	4382–1	71	+	2	10	90S
83	4388(2)	71	–	2	15	25S
84	4390–2(2)	71	+	1	15	25MS
85	4415(1)	71	–	2	5	90S
86	4415(2)	71	–	4	35	35S
87	4418–2–2(2)	71	–	0	30	30S

Table 1 (continued)

No.	Accession	Days to heading	<i>Yd2</i> gene	BYD symptoms severity (0–9)	Net blotch severity (%)	Leaf rust severity (%) and response
88	4530–1(1)	71	–	2	15	55S
89	4530–1(2)	71	+	0	20	55S
90	4756(3)	71	+	0	10	60S
91	4769-1-2(1.1)	71	+	0	12	15MS
92	4769-1-2(2)	71	+	0	8	35MS
93	4769–2	71	+	0	15	10MS
94	4773–2	71	+	1	25	55S
95	4784–1(1.1)	71	–	0	8	40S
96	4784–1(2.1)	71	–	2	10	45S
97	4784–1(2.2)	71	+	0	12	20S
98	4806–1(1)	71	+	0	8	25S
99	4818(1.1)	71	+	2	65	25S
100	4818(1.2)	71	++	1	50	40S
101	4818(1.3)	71	+	2	2	1R
102	4818(2)	71	++	0	50	45S
103	4823–3(1.1)	71	+	1	10	60S
104	4823–3(1.2)	71	+	1	10	45MS
105	4833–2	71	–	4	10	60S
106	4841–2(1.1)	71	+	4	8	5MR
107	4981–1	71	+	7	12	20S
108	64,068–1	71	–	2	25	65S
109	64,105(1.2)	71	+	2	10	80S
110	64,178(1.2)	71	+	1	7	85S
111	64,217–2(1.1)	71	+	3	7	40S
112	64,223(1)	71	+	1	25	45S
113	Gold (CI1145)	71	+	4	15	55S
114	Shenen local (2)	71	+	0	10	20S
115	HB-1307 (EH-1700/F71.B1.63)	71	+	2	8	30S
116	Cross # 41/98	71	+	5	<1	2R
117	Miscal-21	71	+	3	2	2R
118	Bekoji 1(EH1293/F2–18B-11-1-14-18)	71	–	5	2	2R
119	Gold (CI1145)	71	+	5	2	20MS
120	Chelia local (2)	71	–	5	<1	5MR
121	4769-1-2(1.2)	71	+	1	10	30S
122	4769-1-2(2)	71	–	1	2	35S
123	4841–2(1.1)	71	–	2	5	85S
124	4841–2(1.2)	71	+	2	5	85
125	4784–1(2)	71	+	2	2	10S
126	4806–1(2)	71	–	0	85	5MS
127	64,217–2(1.2)	71	–	2	8	10S
128	64,217–2(2)	72	+	2	7	85S
129	Shenen local (1)	72	+	1	10	20S
130	1729.2	72	+	2	5	40MS
131	1820	73	++	1	10	5MR

Table 1 (continued)

No.	Accession	Days to heading	<i>Yd2</i> gene	BYD symptoms severity (0–9)	Net blotch severity (%)	Leaf rust severity (%) and response
132	3988–1(2)	73	–	2	10	25MS
133	4243-1-2 (1)	73	–	0	3	25MS
134	3988–1(3)	74	–	5	5	30S
135	4239-2-2(2)	74	+	0	1	10MR
136	Shenen local (3)	74	–	4	3	35S
137	EH 1847 /F4.2P.5.2 (BEA/IBON 64/91)	74	–	5	1	2R
138	4915–1(1.2)	74	+	1	10	5R
139	4915–1(1.3)	74	+	1	10	10MR
140	3713–2	76	–	0	3	45S
141	1818–1(1)	79	–	0	10	1R
142	1822–1(1)	79	+	0	5	1R
143	1822–1(2)	79	–	2	5	5MR
144	4046–1	79	–	1	3	35S
145	4075-2-3(2)	79	–	5	3	3R
146	HB-42(1)	79	+	7	<1	2R
147	HB-42(2)	79	–	8	1	1R
148	4243-1-2(2)	79	–	5	2	20S
149	4304–2(2)	79	+	7	1	1R
150	Holker	80	+	4	2	1R
151	HB-42(3)	83	+	5	5	2R
152	HB-42(4)	83	–	7	1	<1R
153	1831–2-2(2)	83	+	0	10	2R
154	1818–1(2)	83	+	0	10	5MR
155	HB-42(5)	83	+	7	1	2R
156	HB-42(6)	83	+	7	<1	2R
157	HB-42(7)	83	+	7	<1	1R
158	HB-42(8)	83	–	5	10	5R
159	HB-42(9)	83	–	5	2	3R
160	HB-42(10)	83	–	8	1	1R
161	4388(1)	83	–	5	3	75S
162	EH 1493/F6.32H.3	83	+	7	1	1R
163	Ardu 12	83	+	5	1	1R
164	4784–1(1)	83	–	7	<1	3MR
165	4291–1(1.1)	88	+	0	10	50S

Accessions were arranged in ascending order, with those having similar days to head (DtH) were collected together to simplify comparison; ±, Presence/absence of *Yd2* gene as detected by Ylp marker; BYD severity, assessed based on 0–9 scale (Schaller and Qualset, 1980); CI, Cereal introduction/ plant introduction accession number assigned by USDA; 0–85, percentage disease severity; R, Resistant; MR, moderately resistant; MS, moderately susceptible; S, Susceptible phenotype

1:1000 dilution of the BYDV-PAV polyclonal antibodies (=BYDV-B; Cat No. 140112, Bioreba, Switzerland) was used as the primary antibody. Goat anti-rabbit antibody conjugated to alkaline

phosphatase was used as the probe antibody. Test plants considered positive based on the development of purplish to brown granules in the phloem tissue of the samples following the TBIA test.

Table 2 Differences of BYDV-PAV detectability and movement from point of inoculation in barley accessions with (+ *Yd2*) and without (-*Yd2*) resistant gene when tested by Tissue blot Immunoassay (TBIA) 2–25 days after inoculation

Accession	Accession No. in Table 1	TBIA test results at 2–25 days after inoculation (no. of plants infected/no. of plants tested)						<i>Yd2</i> gene ($\pm$ = Presence/absence of <i>Yd2</i> gene as detected by Ylp marker)
		2	5	10	15	20	25	
1729.2	130	0/6	0/6	6/6	0/6	1/6	3/6	+
1785-1-1	1	0/6	0/6	0/6	0/6	0/6	4/6	+
1831-2-2(1)	10	0/6	0/6	2/6	0/6	0/6	2/6	+
1818-1(1)	141	0/6	0/6	0/6	0/6	0/6	0/6	+
1820	131	0/6	0/6	4/6	0/6	0/6	0/6	+
1836(1)	11	0/6	0/6	0/6	0/6	3/6	0/6	+
3326-1-1	16	0/6	0/6	0/6	0/6	0/6	2/6	+
3412 (1)	17	0/6	0/6	0/6	0/6	0/6	1/6	+
3492-2 (1)	18	0/6	0/6	1/6	1/6	6/6	0/6	+
3675-1(4)	5	0/6	0/6	2/6	1/6	0/6	5/6	+
4228-1-2(2)	31	0/6	0/6	1/6	0/6	1/6	0/6	+
4228-1.2(3)	32	0/6	0/6	0/6	2/6	0/6	1/6	+
4243-1-1	37	0/6	0/6	0/6	0/6	0/6	2/6	+
4253 (2)	39	0/6	0/6	0/6	0/6	0/6	1/6	+
4264 (1)	75	0/6	0/6	0/6	2/6	2/6	4/6	+
4290-2 (1)	76	0/6	0/6	0/6	2/6	0/6	2/6	+
4291-1 (1.2)	42	0/6	0/6	0/6	0/6	0/6	2/6	+
4300-1 (3)	44	0/6	0/6	0/6	0/6	0/6	3/6	+
4304-2 (1)	47	0/6	0/6	0/6	0/6	0/6	3/6	+
4312	46	0/6	0/6	0/6	0/6	0/6	1/6	+
4382-1	82	0/6	0/6	0/6	0/6	2/6	5/6	+
4390-2 (2)	84	0/6	0/6	0/6	0/6	0/6	4/6	+
4530-1 (2)	89	0/6	0/6	0/6	0/6	0/6	3/6	+
4756 (4)	54	0/6	0/6	0/6	4/6	0/6	3/6	+
4765-2	55	0/6	0/6	0/6	0/6	0/6	2/6	+
4767-1-1	56	0/6	0/6	0/6	0/6	0/6	0/6	+
4769-1-2 (1)	91	0/6	0/6	0/6	1/6	0/6	0/6	+
4769-2	93	0/6	0/6	0/6	0/6	0/6	0/6	+
4773-2	94	0/6	0/6	0/6	3/6	5/6	6/6	+
4784-1(2.2)	97	0/6	0/6	0/6	1/6	5/6	2/6	+
4806-1(2)	57	0/6	0/6	0/6	2/6	4/6	0/6	+

Table 2 (continued)

Accession	Accession No. in Table 1	TBIA test results at 2–25 days after inoculation (no. of plants infected/no. of plants tested)						Yd2 gene (± = Presence/ absence of Yd2 gene as detected by Ylp marker)
		2	5	10	15	20	25	
4818 (2)	102	0/6	0/6	0/6	1/6	2/6	0/6	+
4823–3 (1.1)	103	0/6	0/6	0/6	0/6	5/6	6/6	+
4841–2 (1.2)	124	0/6	0/6	0/6	0/6	5/6	2/6	+
4883	59	0/6	0/6	0/6	0/6	2/6	4/6	+
4915–1 (1.3)	139	0/6	0/6	0/6	0/6	1/6	2/6	+
4981–1	107	0/6	0/6	0/6	0/6	3/6	0/6	+
64,016–1–2	61	0/6	0/6	0/6	0/6	0/6	0/6	+
64,105 (1.1)	62	0/6	0/6	0/6	0/6	0/6	2/6	+
64,106–2–1	63	0/6	0/6	0/6	0/6	0/6	1/6	+
64,178 (1.2)	110	0/6	0/6	0/6	0/6	0/6	4/6	+
64,217–2 (2)	128	0/6	0/6	0/6	0/6	2/6	2/6	+
64,223 (1)	112	0/6	0/6	0/6	0/6	0/6	4/6	+
64,226–1	67	0/6	0/6	0/6	0/6	0/6	2/6	+
Gold (IC 1145)	113	0/6	0/6	0/6	0/6	0/6	5/6	+
HB-42(1)	68	0/6	0/6	0/6	0/6	0/6	5/6	+
Shenen local (2)	114	0/6	0/6	0/6	0/6	0/6	3/6	+
Subtotal (+Yd2)		0/282 (0.0%)	0/282 (0.0%)	16/282 (5.7%)	20/282 (7.1%)	49/282 (17.4%)	103/282 (36.5%)	
1822–1(1)	144	0/6	1/6	0/6	0/6	0/6	0/6	–
1838–1	13	0/6	1/6	3/6	0/6	0/6	0/6	–
3167–2	14	0/6	0/6	0/6	0/6	0/6	1/6	–
3324–1–1(1)	15	0/6	0/6	2/6	0/6	0/6	0/6	–
3713–2	140	0/6	0/6	0/6	0/6	3/6	3/6	–
3988–1(2)	132	0/6	0/6	0/6	0/6	0/6	1/6	–
4046–1	144	0/6	0/6	1/6	0/6	2/6	2/6	–
4075–2–3 (2)	145	0/6	0/6	0/6	0/6	0/6	0/6	–
4199 (1)	28	0/6	0/6	0/6	0/6	0/6	2/6	–
4238–2 (1)	36	0/6	0/6	1/6	2/6	0/6	0/6	–
4239–2–1	72	0/6	0/6	4/6	0/6	0/6	1/6	–
4239–2–2 (1)	73	0/6	0/6	0/6	0/6	0/6	1/6	–
4243–1–2 (1)	133	0/6	0/6	0/6	2/6	0/6	1/6	–
4385	74	0/6	0/6	0/6	0/6	0/6	5/6	–

Table 2 (continued)

Accession	Accession No. in Table 1	TBIA test results at 2–25 days after inoculation (no. of plants infected/no. of plants tested)						<i>Yd2</i> gene ($\pm$ = Presence/absence of <i>Yd2</i> gene as detected by Ylp marker)
		2	5	10	15	20	25	
4784–1(2.1)	96	0/6	0/6	0/6	2/6	0/6	1/6	–
4390–1(1.1)	48	0/6	0/6	0/6	0/6	0/6	3/6	–
4415(2)	86	0/6	0/6	0/6	1/6	0/6	6/6	–
4418–2–2(2)	87	0/6	0/6	0/6	1/6	2/6	5/6	–
4833–2	105	0/6	0/6	0/6	0/6	3/6	6/6	–
64,068–1	108	0/6	0/6	0/6	0/6	0/6	0/6	–
Subtotal (– <i>Yd2</i>)		0/120 (0.0%)	2/120 (1.7%)	11/120 (9.2%)	8/120 (6.7%)	10/120 (8.3%)	38/120 (31.7%)	

Results

Evaluation of barley accessions to BYDV-PAV, leaf rust and net blotch

Using the PCR-CAPS primers, expected product size (PS) (331 bp) related to *Yd2* gene was amplified. Out of 165 accessions tested against the *Yd2*-linked markers, 96 (58.2%) of the tested genotypes were confirmed to contain the *Yd2* resistance gene (Table 1). Results of PCR, electrophoresis and UV visualization of purified 48 barley genotypes are shown in Fig. 1.

Data on the association of DtH and BYD symptoms severity was collected (Table 1) to compare if there were differential reactions among accessions. On barley accessions having short DtH (66 days) the disease severity appeared low, and vice versa on accessions having longer DtH (79–83 days). On the basis of DtH and disease symptoms severity score, all the genotypes can roughly be placed in two categories: (i) lines having no BYD symptoms or mild or medium with DtH from 66 to 76 days, and (ii) lines having medium to high symptoms with DtH from 79 to 83 days. At DtH from 66 to 76 days, symptomless lines were recorded in lines both with and without *Yd2* genes, while all symptomless lines at 79–83 DtH were only those lines with *Yd2* gene.

Phenotypic data collected to screen for multiple disease resistant barley showed that 11 lines that contained *Yd2* gene had a disease reaction ranged from highly resistant to moderate (Table 1) for leaf rust and net blotch. Among 11 lines selected as having good level of multiple disease resistance, 6 accessions that showed highest level of reactions were Chelia local, 4304–2(2), 4818(1.3), 1822–1(1), 1831–2–2(2), and 4915–1(1.2). These lines had disease severity and response ranging from <1R to 5R and <1R to 10MR, respectively, for leaf rust and net blotch. Despite all of them contained *Yd2* gene, 2 accessions [1822–1(1) and 1831–2–2(2)] did not develop any BYD-like symptoms, 2 developed low level of yellowing symptom, 1 medium and 1 highest symptoms such as yellowing and stunting. The other 5 accessions [4841–2(1.1), 4915–1(1.3), 1818–1(2), 1820 and 1831–2–2(1)] had moderate level of resistance, with medium (1 accession), low (3 accession) and no (1 accession) BYD symptom developed. Other four accessions (1818–1(1), 1822–1(2), 4075–2–3(2) and 4784–1(1) were negative to *Yd2* gene, but had low severity, and MR and R reactions to leaf rust and net blotch.

Fig. 1 PCR amplifications of *Yd2* gene (PS = 331 bp) conferring resistance to BYDVs from 48 barley accessions. No. 1–48 = tested lines, M = 100 bp DNA size ladder. Accession No. of tested lines (1 to 48), in order, are: 4784(2.1), 4784(2–2), 4806–1(1), 4806–1(2), 4818(1.1), 4818(1.2), 4818(1.3), 4818(2), 4823–3(1.1), 4823–3(1.2), 4833–2, 4841–2(1.1), 4841–2(1.2), 4883, 4915–1(1.1), 4915–1(1.2), 4915–1(1.3), 4981–1, 64,016–1-2, 64,068–

1, 64,105(1.1), 64,105(1.2), 64,106–2-1, 64,178(1.1), 64,178(1.2), 64,217.2(1), 64,217.2(2), 64,223(1), 64,223(2), 64,223(3), 64,226–1, Gold, HB-42, Shenen local(1), Shenen local(2), Shenen local(3), Shenen local(4), HB-1307, Cross 41/98, EH 1493, Ardu 12, Holker, Mis-21, Bekoji 1, EH 1847, IBON 170/03, Local, and Gold

Relationships of virus incubation period and host plant resistance

When barley plants containing resistance gene (+*Yd2*) was inoculated with BYDV-PAV at three leaf stage were tested by TBIA at 2, 5, 10, 15, 20, 25 DAI, BYDV was first detected at 10 DAI. The number of lines infected increased relatively proportionally from 10 DAI to 25 DAI (Table 2). Whilst, when 20 barley accessions without resistance gene (–*Yd2*) were tested by TBIA, BYDV-PAV was detected 5 DAI. BYDV-PAV was not detectable in barley accessions with (+*Yd2*) or without (–*Yd2*) resistance gene 2 DAI. BYDV-PAV could not be recovered from any of the 6 treatments (DAI) in 4 accessions with (+*Yd2*) (accessions 1818–1, 4767-1-1, 4769-1-2 and 64,016–1-2) and two accessions without (–*Yd2*) (accessions 4075-2-3 and 64,068–1). In general, BYDV-PAV detectability increased from 10 DAI to 25 DAI in lines/accessions with *Yd2* gene, although it was inconsistently increased with the highest being recorded at 25 DAI, followed by 10 DAI (Table 2).

Discussion

Ethiopian barley is considered highly diversified (Harlan 1969; Vavilov 1951) and landraces form the major genetic resources of cultivated barley in the country having several traits of agronomic importance (Abebe 2006). The two BYDV-PAV resistant major genes (*Yd2* and *Yd3*), for instance, exclusively identified from the Ethiopian barley (Niks et al. 2004; Rasmusson and Schaller 1959) and have since been transferred to several spring and winter barley cultivars and breeding

lines across the Globe (Burnett et al. 1995). However, this potential had not yet been exploited in barley improvement programs in the country. In this study, *Yd2* gene was identified for the first time in significant proportion (58.2%) of barley genotypes tested using marker PCR (Table 1). This proportion is very high, and in agreement with the findings of Qualset (1975) who reported that the Ethiopian barley collections from high elevations had a much higher frequency of BYD resistance than barleys from the lower areas. The fact that barley collections used in this study were from higher altitudes between 2500 and 3300 masl, and BYD severity and incidence in Ethiopia was more pronounced at same altitude range (Agranovsky 1986; Agranovsky et al. 1985; Bekele et al. unpublished; Bekele et al. 2001, 2003; Yusuf et al. 1992) explains the high proportion of BYDV resistant genotypes from Ethiopia. Based on this fact it is likely to assume that the ecological conditions at higher elevations in Ethiopia are more conducive for both the viruses and their local clones of respective aphid vectors for BYD epiphytotic development, which in turn exerted natural selection pressure geared up toward resulting abundant resistant phenotypes. Hence, this research findings further supported the fact that Ethiopia is rich gene pool for barley where BYDV resistant gene is readily identified from landrace collections and genetic resistance can be easily employed to reduce the effects of the disease. BYD is currently an economic disease of high land grown elite barleys, and the identified resistant genotypes are a good resource for future barley improvement program targeting BYDVs in Ethiopia. As shown in Table 1, some *Yd2* possessing accessions had higher BYD severity than the genotypes that lack this gene. This result

may be interpreted in terms of occurrence of a resistance reaction called ‘Tolerance’ that permits plants to withstand high disease pressure with low yield loss.

A study to monitor the association of days to heading and development of BYD in accessions with (+*Yd2*) and without (-*Yd2*) resistant gene revealed that severe disease symptoms developed in lines having longer days to heading than lines having shorter days to heading. At 83 and 88 DtH, severe BYD symptoms developed in lines both with (+*Yd2*) and without (-*Yd2*) resistant gene. However, the proportion (47%) of lines having severe symptoms was higher for those lines without resistant gene (-*Yd2*). In most of the lines having DtH of 66–69 (42%), the symptoms were mild or absent. The general trend showed that as the DtH increased from 66 (shortest) to 88 (longest), disease severity and incidence increased proportionally, regardless of whether the resistant gene (*Yd2*) is present or not, assuming that the environment is similar. Two possible explanations can be suggested: (i) growth rate may affect the expression of BYD symptoms; or (ii) the level of tolerance conferred by *Yd2* gene may be modified by host genotype and environmental factors. Hayes et al. (1971) found significant positive correlation between levels of tolerance expressed by the parents and progeny with growth rate, suggesting that environmental or genetic factors which delay heading diminish the expression of BYDV tolerance. Similarly, research results of Jones and Catherall (1970) suggested that barley varieties were most tolerant to infection with barley yellow dwarf virus (BYDV) when they grew rapidly, whether the rate of growth was determined by manipulation of the environment or by the innate genetic constitution of the host. Having experienced difficulty in recovering late maturing segregates, Jones and Catherall (1970) suggested that the *Yd2* gene operates by retarding virus multiplication, thus allowing the virus to reach higher concentrations over a longer period of time.

In an attempt to adopt affordable and cheaper alternative methods of evaluation to differentiate resistant and susceptible barley genotypes using TBIA showed that BYDV-PAV was readily detectable five days after inoculation in stems of susceptible (-*Yd2*) than resistant (+*Yd2*) lines. This finding is in agreement with reports of previous studies on other crops that the relative restriction of virus movement in resistant cultivars has been documented for a number of sap transmissible viruses such as *Cucumber mosaic virus* (CMV) in pepper (Doufour et al. 1989; Nono-Womdim et al. 1991)

and *Maize dwarf mosaic virus* (MDMV) in maize (Law et al. 1989). Previously, Jensen (1973) also reported that BYDV moves from an inoculated leaf more readily to roots in susceptible than in resistant cereals. Similarly, Makkouk and Ghulam (1992) reported differences in the rate of BYDV movement from the inoculated leaf to the root in resistant and susceptible barley genotypes. Makkouk et al. (1994) indicated that multiplication and/or movement of barley yellow dwarf virus was retarded in resistant (R) than in susceptible (S) barley genotypes. They showed that DAS-ELISA testing of root extracts from 41 genotypes three or four days after inoculation at the one leaf stage resulted in reliable differentiation of susceptible from resistant plants. Further testing of barley plants inoculated at the two or three leaf stage using tissue-blot ELISA on nitrocellulose membranes revealed that the virus was detected 4–6 days after inoculation in the phloem vessels of the growing points of the susceptible genotypes, but not of resistant genotypes, indicating the method is rapid and inexpensive technique for screening for BYDV resistance in barley. It is to be recalled from Table 2 that BYDV-PAV was not uniformly and consistently recovered from TBIA testing five DAI and onwards among most accessions without resistant gene (-*Yd2*), and yet the virus was not recovered in two such lines. In the case of barley accessions with resistant gene (+*Yd2*), BYDV-PAV was recovered most frequently at 25 DAI, supporting the report that the *Yd2* resistant gene suppress/retard virus movement and/or multiplication. As in the case of accessions without *Yd2*, BYDV-PAV was not recovered from four lines. Absence of positive TBIA result in this case may be attributed to either inherent genetic background of the host that completely resist the virus conferred by the same gene or other gene(s) (major and minor) operating concurrently or infection escape or the efficiency of TBIA in detect low virus titer. Similarly, absence of positive TBIA reactions in two of *Yd2* negative barley accessions may also imply the presence of other effective gene(s) other than *Yd2* or infection escape due to lack of uniform application of viruliferous aphid vectors. In general, as in the case of previous studies, the result based on virus movement gave good indications in selecting promising barley lines in preliminary screening. Thus, to use the method solely to develop BYDV resistant genotypes, supporting data such as yield and associated agronomic traits may be taken into consideration.

It is interesting to note that some barley genotypes found resistant to BYD were also resistant to leaf rust and

net blotch. Cultivars resistant to multiple diseases are scarce since breeding for multiple disease resistance is a difficult and lengthy task (<https://www.researchgate.net/publication/265820772>). Luckily, eleven barley genotypes possessing multiple disease (leaf rust, net blotch and BYDV) resistance were successfully identified in the present study. Such multiple disease resistance is a desirable trait of good agricultural importance to be incorporated in elite barley genotypes through backcross breeding. In most cases, these results were hardly attainable, and signifies the great diversity of, and characteristic feature and quality of, Ethiopian barley landrace collections. Thus, barley breeders should take advantage of this opportunity in producing multi-disease resistant/tolerant barley genotypes in an agronomically enhanced genetic background in Ethiopia and beyond.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights This research does not contain any studies with human participants or animals performed by any of authors.

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