Advances in Crop Science and Technology

Woldeab et al., Adv Crop Sci Tech 2015, 3:2
http://dx.doi.org/10.4172/2329-8863.1000164

Research Article Open Access

Virulence Spectrum of *Puccinia hordei* of Barley in Western and Central Highlands of Ethiopia

Getaneh Woldeab[1], Endale Hailu[1]*, Teklay Ababa[2] and Teklu Negash[1]

[1]*Ethiopian Institute of Agricultural Research, Ambo Plant Protection Research Center, P.O. Box 37, Ambo, West Shewa, Ethiopia*
[2]*Tigray Agricultural Research Institute, Alamata Agricultural Research center, P.O. Box 56, Alamata, Ethiopia*

Abstract

Virulence surveys of Puccinia hordei of barley were conducted in the main and off crop seasons of 2010/11 and 2011/12 in West Shewa, Wellega (western part of the country) and Arsi (central part of the country) zones of Oromiya region, Ethiopia to determine the virulence spectrum of the pathogen, and identify the effective resistance genes to the pathotypes. In the two crop seasons, 56 leaf (brown) rust samples in the main and 32 in the off-season were collected. From each barley field, single pustule descent spores were multiplied and inoculated onto the seedlings of 12 leaf rust differentials carrying Rph1 - Rph12 genes to designate the pathotypes. A total of 88 leaf rust isolates were processed and based on infection phenotype on the resistance genes, 7 pathotypes (ETPh6631, ETPh6611, ETPh6671, ETPh7671, ETPh7631, ETPh7611 and ETPh7651) were identified. The most frequently isolated pathotype was ETPh6631 with 43.2% followed by ETPh6611 with 19%. Moreover, virulence spectrum of *P. hordei* pathotypes identified in this study was diverse. Resistance genes with Rph1 (Sudan), Rph4 (Gold), Rph8 (Egypt4), Rph9 (Hor 2596), Rph11 (Clipper BC68) and Rph12 (Triumph) were non-effective to all pathotypes identified whereas genes Rph5 (Magnif), Rph6 (Bolivia) and Rph10 (Clipper BC 8) were effective to 26.1, 73.9 and 78.4% of the isolates, respectively. Virulence against Rph2 (Peruvian), Rph3 (Estate) and Rph7 (Cebada Capa) was absent. Therefore, the effective major genes to the existing leaf rust populations could be utilized as sources of resistance in the barley breeding program.

Keywords: Barley; *Hordeum vulgar;* Leaf/brown rust; *Pathotypes*; *Puccinia hordei*; Pathotype specific resistance

Introduction

Barley (*Hordeum vulgarae*) is one of the most important staple food crops in the highlands (2,000-3,000 meters above sea level) and ranks fifth in area and production among the cereals in Ethiopia. It was cultivated on 1.02 million hectares and total annual grain production in 2012/13 cropping season was about 1.78 million tons [1]. Barley is produced mainly in Shewa, Gojam, Bale, Arsi, Gondar, Wello, Wellega and Tigray zones [2]. It is grown in main rainy, residual and belg/off-seasons, the largest production being in the main rainy season (June– October). Productivity of barley in Ethiopia is low (only about 1.8 ton ha^{-1}) as compared to some major barley producing countries. Diseases are one of the main biotic constraints to barley production [2,3].

Barely leaf rust caused by a fungus *Puccinia hordei* is prevalent on cultivated and indigenous wild barleys. It is particularly important in areas where the crop matures late, occurring extensively in barley areas of the Eastern, Mid-Western United States and in North Africa, Europe, New Zealand, Australia, some part of Asia and in the Andes region of south America [4,5]. The disease was first reported in Ethiopia by Stewart and Dagnatchew [6]. Since then, it has become widespread in all barley growing regions of Ethiopia [7]. It is favoured by a relatively warm and moist climate [8]. Barley leaf rust causes serious yield losses in the countries of North Africa and in Pakistan [9]. Losses due to this disease reached 23% on a susceptible variety Trompllo at Ambo Plant Protection Research Centre experimental field [10] and 28% on white seeded barley variety on farmer's fields at Tikur Inchini and Shenen districts of West Shewa zone [11].

The fungus is a heterocious pathogen with Hordeum species as primary hosts and *Orinthgalem umbellatum* as alternate host [12]. It completes its life cycle on alternate host and other species. The alternate host also supports genetic-recombination of the leaf rust fungus resulting in the evolution of broad spectra of the pathogen variability [13]. At present, many of the leaf rust resistance genes derived from barley have limited value for plant breeding because *P. hordei* pathotypes with virulence on them have evolved [14]. More than 52 physiological pathotypes of *P. hordei* have been reported to infect barley in the world [15]. Earlier investigations of barley leaf rust isolates indicated the presence of pathotypes 77 and 184 in a few locations of Ethiopia [3]. In a recent study, seven pathotypes were identified from 381 isolates collected from the barley growing highlands of Gojam, Shewa and Bale zones [16]. However, the study made in 2006 did not cover other major barley growing areas like west Shewa, Wellega and Arsi zones. Therefore, these surveys were conducted to determine the virulence spectrum of *P. hordei* pathotypes occurring in three zones of Oromiya region, Ethiopia.

Materials and Methods

Barley leaf rust surveys were conducted in 2010/11 and 2011/12 cropping seasons. The major barley growing areas of Toke Kutaye district in west Shewa; Jima Arjo, Abay Chomen, Jima Geneti and Diga Leka districts in Wellega (western part of Ethiopia) and Robe, Sude, Hitosa and Meraro districts in Arsi zone (central part of Ethiopia) were surveyed in the main (September – October) seasons. Barley fields

***Corresponding author:** Endale Hailu, Ethiopian Institute of Agricultural Research, Ambo Plant Protection Research Center, P.O. Box 37, Ambo, West Shewa, Ethiopia, Tel: 25191363779; E-mail: sebhailuabera@yahoo.com

Received September 20, 2014; **Accepted** March 25, 2015; **Published** March 27, 2015

Citation: Woldeab G, Hailu E, Ababa T, Negash T (2015) Virulence Spectrum of *Puccinia hordei* of Barley in Western and Central Highlands of Ethiopia. Adv Crop Sci Tech 3: 164. doi:10.4172/2329-8863.1000164

were also assessed for leaf rust infection in the off season (December – January) in Wellega zone. During the surveys, rust infected leaf samples were collected from the barley fields and the rust spores from each field were inoculated with an atomizer on the seedlings of susceptible cultivar L94 in the greenhouse at Ambo Plant Protection Research Centre to get isolated pustules. Each pustule/isolate extracted from the leaf rust population to represent a field was multiplied until sufficient spores were collected by tapping the rusted leaves in the test tube/ watch glass and this was inoculated on the differential hosts.

Cereal introduction/plant introduction accession number assigned by United States Department of Agriculture (USDA).

Twelve barley leaf rust differential hosts indicated on Table 1 and a universally susceptible check L94 were used to analyse *Puccinia hordei* populations collected from the three zones. Barley seeds were grown in greenhouse at temperature of 20-24°C in suitable 10 cm diameter clay pots filled with the ratio of 2:1:1 soil: sand: farm yard manure. Inoculation was carried out when the seedlings were between 5 and 10 cm long or at two leaf stages when the first leaf was fully extended. Before inoculation, the leaf surfaces were gently rubbed with moistened fingers to remove the outer waxy coating of the leaves and then the seedlings were sprayed/misted with distilled water.

Urediniospores of each isolate were suspended in distilled water with two drops of tween 20 as spreading and adhering agent. The suspension was sprayed onto seedlings of the differentials using atomizers. After that, the plants were moistened with distilled water and placed in an incubation chamber for 16-20 hr dark period at 20°C. The seedlings were then taken out onto the growth room where florescent lighting was provided for 16 hours at 20°C and RH of 60 - 70%. Within 7 to 10 days after inoculation, data on the infection types were recorded.

The infection types of barley leaf rust were read for each differential host using Levine and Cherewick [17] in 0-4 scoring scale. The infection types 0, 0; 1 and 2^{+} were taken as incompatible/ resistance (low) and 3^{-} and 4^{++} were taken as compatible/susceptible (high) reactions. Octal race designation system was used to name the *pathotypes*. In this system, *pathotypes* are determined by adding values that correspond to each differential to which the isolate was virulent [18].

Differential hosts	CI/PI number	Resistance gene	Octal value
Sudan	CI6489*	*Rph1*	1
Peruvian	CI935	*Rph2*	2
Estate	CI34102	*Rph3*	4
Gold	CI1145	*Rph4*	10
Magnif	CI13806	*Rph5*	20
Bolivia	CI1257	*Rph6 +Rph2*	40
Cebada Capa	CI6193	*Rph7*	100
Egypt 4	CI6481	*Rph8*	200
Hor 2596	CI1243	*Rph9*	400
Clipper BC8	-	*Rph10*	1000
Clipper BC67	-	*Rph11*	2000
Triumph	PI2668 180	*Rph12*	4000

Table 1: *Puccinia hordei* differential hosts with CI/PI number, resistance genes and octal values.

For example: ETPh6611 is virulent on *Rph1+Rph4+Rph8+Rph9+ Rph11+Rph12= 1+10+200+400+2000+4000=6611* and ETPh means Ethiopian *Puccinia hordei.*

Results and Discussion

Pathotype and virulence surveys of *Puccinia hordei* were carried out in one district of West Shewa; four districts of Wellega and four districts of Arsi zones during the main season of 2010/11 and 2011/12. In the first main season, 40 isolates and in the second, 16 isolates collected from the three zones were processed and seven pathotypes were identified (Table 2). In the two off-seasons in the four districts of Wellega zone, 32 isolates were collected and analyzed and four pathotypes (ETPh6611, ETPh6631, ETPh6671 and ETPh7671) were isolated. From the whole of 88 leaf rust samples, seven pathotypes (ETPh6631, ETPh6611, ETPh6671, ETPh7671, ETPh7631, ETPh7611 and ETPh7651) were identified. The numbers of pathotypes isolated were 5 5, and 6 from Wellega, West Shewa and Arsi zones, respectively. In the off-season, of the seven, pathotypes ETPh7631 and ETPh7651 were absent. In the main and off seasons in Wellega, four pathotypes each were identified, of which three of them were identical while the other two were different. The off season barley comes right after the main season barley matures, so late maturing varieties as well as volunteer barleys can carry the rust disease from the main to the off season barley. The similarity of the pathotypes might be due to the movement of

Year	Season	Zone	No. of isolates tested	No. of pathotypes identified	Pathotypes
2010/11	1*	West Shewa	13	3	ETPh6631, ETPh7631, ETPh6671
	1	Arsi	21	6	ETPh6611, ETPh6631, ETPh6671 ETPh7611, ETPh7631, ETPh7671
	1	Wellega	6	3	ETPh6611, ETPh6631, ETPh7671
Sub			40	6	
2011/12	1	West Shewa	3	2	ETPh7611, ETPh7651
	1	Arsi	8	4	ETPh6611, ETPh6631, ETPh6671, ETPh7671
	1	Wellega	5	2	ETPh6611, ETPh7611
Sub			16	6	
Total			56	7	
2010/11	2	Wellega	26	3	ETPh6631, ETPh6671, ETPh7671
2011/12	2	Wellega	6	1	ETPh6611
Sub total			32	4	
Grand total (1+2)			88	7	ETPh6611, ETPh6631, ETPh6671, ETPh7611, ETPh7631, ETPh7651, ETPh7671

*1 is main season, 2 off season

Table 2: *Puccinia hordei* pathotypes identified from barley growing areas of West Shewa, Wellega and Arsi zones, 2010/11-2011/12 seasons.

spores from one to the other season. The number of pathotypes in Arsi zone was higher than the races in West Shewa and Wellega. This could probably be because in Arsi zone the diversity of local and improved barley varieties grown is high. Moreover, the pathotypes identified in the two different seasons were similar indicating that carry over of the pathogen from one to the other season occurs in the areas. Besides, many of the pathotypes found during this study were similar with those identified from Bale, and north west Shewa zone of Oromiya region as well as from Gojam and north east Shewa zones of Amhara region in 2003 and 2004 [16]. Thus, earlier and this studies demonstrate the heterogeneity of the leaf rust pathogen in Ethiopia.

Virulence spectrum and frequency of *Puccina hordei* pathotypes in the three zones and in the main and off-seasons of 2010/11 – 2011/12 are shown in Table 3. Thirty-eight (43.2%) of the 88 isolates were pathotype ETPh6631. It was distributed in all three zones in both seasons and years. In the first main season, pathotype ETPh6631 was isolated from all three zones, while in the second, it was observed in Arsi zone. In the off season of 2010/2011 pathotype ETPh6631 was recorded, but not in the second season. This pathotype was not isolated in the virulence study of *P. hordei* in 2003 and 2004. This pathotype was identified for the first time in Ethiopia, however, theses zones were not covered in the previous study. ETPh6611 was the third frequently isolated pathotype in the previous study. Pathotype ETPh6671 was not isolated in earlier studies. Except ETPh6631 and ETPh6671, the rest of the pathotypes were isolated in Ethiopia before. The second commonly found pathotype was ETPh6611 with a frequency of 17 isolates (19.3%), followed by pathotype ETPh6671 with 14 isolates (15.9%). ETPh6611 was isolated from Wellega and Arsi, while ETPh6671 was from west Shewa and Arsi zones. The rest of the pathotypes had distribution below 10%, with a minimum frequency of 2.3% for pathotype ETPh7651. This pathotype was identified twice in the main season of 2011/12 in west Shewa zone. Previously, Woldeab et al. reported that pathotype ETPh6611 and ETPh7651 were the third and the fourth frequently isolated pathotypes, respectively in 2003 and 2004 cropping seasons [16].

Virulence spectrum of *Puccinia hordei* pathotypes identified from the three zones was diverse (Table 3). The least virulence was observed for pathotype ETPh6611, while the highest was recorded for pathotype ETPh7671. Six and nine out of the 12 *Puccinia hordei* resistant genes were defeated by these pathotypes, respectively. The second highest virulence spectrum was recorded for pathotypes ETPh6671, ETPh7631 and ETPh7651 with virulence to eight resistant genes.

Among the resistant genes, *Rph1* (Sudan), *Rph4* (Gold), *Rph8* (Egypt4), *Rph9* (Hor 2596), *Rph11* (Clipper BC68) and *Rph12* (Triumph) were non-effective to the 88 isolates tested. *Rph5* (Magnif), *Rph6* (Bolivia) and *Rph10* (Clipper BC8) were also ineffective to 73.9, 26.1 and 21.6% of the isolates, while *Rph2* (Peruvian), *Rph3* (Estate) and *Rph7* (Cebada Capa) genes were effective/resistant to all isolates identified (Table 3).

Races	Virulence/avirulence of the pathotypes	Frequency (No. & percent)
ETPh6631	*Rph*1, 4, 5, 8, 9, 11, 12 / 2, 3, 6, 7, 10	*38* (43%)
ETPh6611	*Rph*1, 4, 8, 9, 11, 12 / 2, 3, 5, 6, 7, 10	17 (19.3%)
ETPh6671	*Rph*1, 4, 5, 6, 8, 9, 11,12 / 2, 3, 7, 10	14 (15.9%)
ETPh7671	*Rph*1, 4, 5, 6, 8, 9, 10, 11, 12 / 2, 3, 7	7 (7.9%)
ETPh7631	*Rph*1, 4, 5, 8, 9, 10, 11,12 / 2, 3, 6,7	6 (6.8%)
ETPh7611	*Rph*1, 4, 8, 9, 10, 11, 12 / 2, 3, 5, 6, 7	4 (4.5%)
ETPh7651	*Rph*1, 4, 6, 8, 9,10, 11, 12 / 2, 3, 5, 7	2 (2.3%)

Table 3: Virulence and avirulence formula and the frequency of the seven pathotypes identified on the 12 *Rph* genes from west Shewa, Wellega and Arsi zones, 2010/11 – 2011/12 cropping seasons.

In earlier studies, *Rph3* (Estate) and *Rph7* (Cebada Capa) genes were also effective to 381 leaf rust isolates collected from different regions and production systems of Ethiopia [16]. Before 2006, similar results were reported by Alemayehu from Ethiopia and Park from Australia [7,19]. However, there is a report of virulence to *Rph3* from Europe [20] pathotypes virulent for *Rph7* have been reported in Israel [21], but not in Europe and Australia [14]. Furthermore, gene *Rph9* has been deployed in commercial cultivars worldwide, [22] but this gene has been defeated by the pathogen in many locations, including North Africa, the Middle East, and Australia [19,23]. At present, most of the Ethiopian barley landraces are susceptible to leaf rust, and one such landrace, Abyssinian, known to contain *Rph9*, is very susceptible.

The present study demonstrated pathogenic heterogeneity of the barley leaf rust populations in West Shewa, Wellega and Arsi zones. If barley with known major gene resistance were to be deployed, then the use of *Rph3* and *Rph7*, and perhaps *Rph2*, would be expected to give the best protection against leaf rust in Ethiopia.

References

1. CSA, Central Statistical Authority (2013) Agricultural sample survey 2012/2013, Report on area and production of crops meher season. Statistical Bulletin No 532, 1:15 CSA, Addis Ababa.
2. Lakew B, Gebre H, Alemayehu F (1996) Barley production and research. In: Gebre H, van Leur J (eds) Barley research in Ethiopia: past work and future prospect. IAR, Addis Ababa pp. 1-8.
3. Yitbarek S, Bekele H, Getaneh W, Dereje T (1996) Disease survey and loss assessment studies on barley. In: Hailu Gebre and Joop van Leur (eds) Barley research in Ethiopia, past work and future prospects. pp. 105-115, Addis Ababa, Ethiopia.
4. Dickson JC (1956) Diseases of field crops. Mc-Crow-Hill Publishing Co.Ltd, Bombay-New Delhi, India.
5. Mathre DE (1987) Compendium of barley diseases. American Phytopathological Society, pp. 78.
6. Stewart RB, Yirgu D (1967) Index of plant diseases in Ethiopia. College of Agriculture Haile Selase I. University Experimntal Station Bulletin No, 30.
7. Alemayehu F (1995) Genetic variation between and within Ethiopian barley landraces with emphasis on durable disease resistance. The Netherlands: Wageningen Agricultural University. PhD Thesis.
8. Bokelman HE, Sharp EI, Sands DC, Schoren AL, Mathre DE (1981) Field manual of common barley diseases including a section on Breeding disease resistant barley. Department of Plant Pathology, Montana State University, Bozeman, Montana 59717, USA. pp. 3-56.
9. Stubbs RW, Prescott JM, Saari EE, Dubin HJ (1986) Cereal disease methodology manual. CIMMYT, Mexico. pp. 45-46.
10. Getaneh W (1998) Yield loss due to leaf rust on barley at different sowing dates. Pest Management Journal of Ethiopia 1: 79-84.
11. Getaneh Woldeab, Fekadu Alemayehu (2001) On-farm yield loss due to leaf rust (Puccinia hordei Otth) on barley. Pest Management Journal of Ethiopia. 5: 29-35.
12. Clifford BC (1985) Barley leaf rust. In: Roelfs AP, Rashnell WH (eds), The cereal rusts II Disease, distribution, epidemiology and control. Orlando Press, Baton Rouge, pp. 173-305.
13. Anikster Y, Abraham C, Greenberger Y, Wahl I (1971) A contribution to the taxonomy of Puccinia brown leaf rust of barley in Israel. Israel journal of Botany 20: 1-12.
14. Dreiseitl A, Steffenson BJ (2000) Postulation of leaf rust resistance genes in Czech and Slovak barley cultivars and breeding lines. Plant Breeding 119: 211-4.

15. Fadeev UM (1977) Metodicheskie recommendasi po izucheniyu racavova sostava vozbuditelei rrzavchini hlebnih zlakov, Moskva pp. 132-140.

16. Woldeab G, Fininsa C, Singh H, Yuen J (2006) Virulence spectrum of Puccinia hordei in barley production systems in Ethiopia. Plant Pathology 55: 351-357.

17. Levine MN, Cherewick WJ (1952) Studies on Dwarf Leaf Rust of Barley. Department of Agriculture. (USDA Technical Bulletin 1056), Washington: United States.

18. Gilmour J (1973) Octal notation for designating physiological races of plant pathogens. Nature 242: 260.

19. Park RF (2003) Pathogenic specialization and pathotype distribution of Puccinia hordei in Australia, 1992-2001. Plant Disease 87:1311-6.

20. Niks RE, Walther U, Jaiser H, Martinez F, Rubiales De, et al. (2000) Resistance against barley leaf rust (Puccinia hordei) in West European spring barley germplasm. Agronomie 20: 769-82.

21. Brodny U, Rivadeneira M (1996) Physiologic specialization of Puccinia hordei in Israel and Ecuador: 1992–94. Canadian Journal of Plant Pathology 18: 375-8.

22. Brooks WS, Griffey CA, Steffenson BJ, Vivar HE (2000) Genes Governing Resistance to Puccinia hordei in Thirteen Spring Barley Accessions. Phytopathology 90: 1131-1136.

23. Yahyaoui AH, Sharp EL (1987) Virulence spectrum of Puccinia hordei in North Africa and the Middle East. Plant Disease 71: 597-8.

Citation: Woldeab G, Hailu E, Ababa T, Negash T (2015) Virulence Spectrum of *Puccinia hordei* of Barley in Western and Central Highlands of Ethiopia. Adv Crop Sci Tech 3: 164. doi:10.4172/2329-8863.1000164