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Virulence Spectrum of *Phytophthora infestans* and Spatial Distribution of Physiological Races in Northwestern Ethiopia

Esmelealem Mihretu[1*], Wassu Mohammod[1], Bekele Kassa[2] and Hannele Lindqvist-Kreuze[3]

[1]*School of Plant Sciences, Haramaya University, P.O.Box 138, Dire Dawa, Ethiopia;*
[2]*Ethiopian Institute of Agricultural Research, Holleta Agricultural Research Center, Holleta, Ethiopia;*
[3]*International Potato Center (CIP), Apartado 1558, Lima 12, Peru*
**corresponding author email: esmelealemm@gmail.com*

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ድንች ለምግብና ለአመጋግብ ዘዴ ዋስትና በተለይም የምግብ ዋስትና ችግር ባለበት አካባቢ ዋነኛ ምግብ ነው፡፡ ምንም እንኳን ድንች ከፍተኛ ጠቀሜታ ያለው ቢሆንም ፋይቶፍቶራ ኢንፌስታንስ በሚባል ተዋስያን አማካኝነት የሚከሰተው የድንች ምች በሽታ ከፍተኛ ጉዳት በማድረስ ለድንች ማምረት ትልቅ ማነቆ ነው፡፡ ጥናቱ በሰሜን ምዕራብ ኢትዮጵያ ዋና ዋና የድንች አብቃይ በሆኑ አካባቢዎች ላይ የፋይቶፍቶራ ኢንፌስታንስ በሽታ አምጭ የዝርያ ዓይነቶችን እና ስርጭታቸውን ለመለየት በ2018 የተካሄደ ነው፡፡ በአጠቃላይ 74 ናሙናዎች ተሰብስበው በድንች ቁርጥራጮች ላይ በማባዛት በብላክስ በሽታ ተቋቋሚ የድንች ዘረመሎች ላይ በሽታ አምጭነታቸው ተገምግሟል፡፡ ናሙናዎቹ በሽታውን በሚቋቋሙ ዘረመሎች ላይ ተሞክረው በአሳዩት አፀፋዊ መልስ መሠረት 6፣ 11 እና 16 የተለያዩ ዓይነት በሽታ አምጭ ዝርያዎች በአዊ፣ በደቡብ ጎንደር እና በምዕራብ ጎጃም በቅደም ተከል መሠረት እንደሚገኙ አሳይቷል፡፡ የሻኖን የአይነት ብዛት መለኪያ ውጤት ለሁሉም ናሙናዎች በአጠቃላይ 0.8 ሲሆን ለአዊ፣ ለደቡብ ጎንደር እና ምዕራብ ጎጃም ዞኖች ለየራሳቸው ደግሞ 0.75፣ 0.84 እና 0.92 በቅደም ተከተላቸው መሠረት ነው፡፡ በአጠቃላይ 74ቱም ናሙናዎች የሦስቱም ዞኖች ፑል ተደርገው ሲታይ 27 የተለያዩ ዓይነት በሽታ አምጭ ዝርያዎች እንዳሉ ተለይተዋል፡፡ ምንም አይነት የዝርያ ውስብስብነት የሌለው ናሙና በደቡብ ጎንደር እና ምዕራብ ጎጃም ዞኖች የተገኘ ቢሆንም ከፍተኛ የዝርያ ውስብስብነት 10 የበሽታ አምጭነት ነገር ያላው ዝርያ በደቡብ ጎንደር ተገኝቷል፡፡ በሽታውን ከሚቋቋሙ ዘረመሎች ውስጥ R3, R5, R8 እና R9 አጠቃላይ ከታየው ልዩነት ከፍተኛ አስተዋፅኦ ያበረከቱ ሲሆን R9 እና R5 በጣም ውጤታማ የነበሩ 95% እና 92% የሚሆኑትን ናሙናዎች በቅደም ተከተል መቋቋም የቻሉ ዘረመሎች ናቸው፡፡ በጠቅላላው ዋና ተቋቋሚ ዘረመል የያዙ የድንች ዝርያዎችን በመጠቀም በሽታውን መከላከል አዳጋች ነው፡፡ ምክንያቱም ሁሉንም ናሙናዎች መቋቋም የቻለ ዘረመል ባለመኖሩ እና ውስብስብ በሽታ አምጭ የዝርያ ዓይነቶች በአካባቢው ተሰራጭተው ስለሚገኙ፡፡

Abstract

Despite the importance of potato the late blight disease, caused by the oomycete **Phytophthora infestans** *is most destructive disease for potato production. The study was conducted to identify virulent races and spatial distribution of* **Phytophthora infestans** *populations in major potato growing areas of northwestern Ethiopia in 2018. Seventy-four isolates (samples) were collected and multiplied on potato tuber slices and virulence was assessed on Black's potato differentials. Isolates reaction to differentials revealed 6, 11, and 16 race types at Awi, South Gondar and West Gojam, respectively. Shannon diversity index was 0.80 for the entire isolates but it was 0.75, 0.84 and 0.92 for isolates collected from Awi, South Gondar and West Gojam, respectively. Tewnty-seven physiological races were detected in the pooled population when the 74 isolates from the three populations were combined. Absence of race complexity was found in isolates of South Gondar and West Gojam while the highest race complexity with virulence factors of 10 was found in South Gondar. Differentials, R3, R5, R8, and R9 had larger contribution to the total variability of isolates and R9 and R5 genes were most effective withstood 95% and 92% of isolates. It is concluded that potato varieties resistant to major genes are hardly possible to be used as disease management option due to the absence of R genes resistant to all isolates, the complexity, and distribution of races in the region.*

Introduction

Despite the importance of potato the late blight disease, caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, is most destructive disease for potato production (Kyamanywa *et al.,* 2011). Ethiopia has 70% of the country's arable land potentially suitable to potato cultivation (Yilma 1991). Potato is grown on more than 296,578 hectares and a total of 3.66 million tons with an average yield of 12.66 t ha^{-1} is produced (CSA 2016). The northwestern Ethiopia mainly the Amhara regional state is one of the major potato growing areas in the country accounting for 25% of the potato-growing farmers. In this region, a total of 747,593.6 tons on 80,252.9 hectares of land with average 9.32 t/ha was produced (CSA 2016). However, production and productivity of potato is lower than the national average yield, which is constrained by many biotic and abiotic stresses in the region of which late blight, a disease caused by *Phytophthora infestans*, is seriously threatening the production (Kassa *et al.,* 2002). The disease is known as the most destructive pathogen of potato, particularly in areas experiencing moderate temperature and high humidity (Yoshida *et al*. 2013).

The annual worldwide potato crop loss due to late blight was estimated at $6.7 billion (Nowicki et al. 2011). In Ethiopia the loss due to the disease is estimated to range between 65-70% and complete crop failures are frequently reported (Bekele and Gebre-Medhin 2000; Denitsa and Naidenova 2005). Efforts have been made to develop more than 30 potato varieties resistant to *P. infestans* over the last three decades by the National Potato Research Program under the Ethiopian Institute of Agricultural Research, Regional Agricultural Research Institutes, and Universities as disease management option (Getachew *et al.* 2016). However, cultivars lost their resistance soon after dissemination to farmers. As a result, the commercial potato farmers in Ethiopia mostly rely on fungicide applications to control *P. infestans* (Habtamu *et al.* 2012). The potato varieties recommended for different agro-ecologies have become susceptible to late blight for losses of resistance in varieties (Stewart *et al*. 2003; Wastie 1991), varying number of major R-genes and varieties grown where the environment is not favorable for the pathogen but become susceptible when grown in other agro-ecologies (Beukema and Van Der Zaag 1979).

Determining the characteristics of the local pathogen population can help in designing efficient disease management strategies, such as the rationale of the deployment of race-specific R-genes in breeding programs. This is because, the varieties developed for one agro-ecology may be distributed and grown in other agro-ecologies under the same management situations for which the breeders did not develop for that agro-ecology and showed lack of adaptation and/or resistance. Moreover, the races of the pathogen can be dispersed anywhere in the country as it cannot restricted to one region (Drenth et al. 1995; Goodwin and Sujkowski 1995) and make cultivars susceptible to late blight disease though initially evaluated as resistant. A large body of evidence has been gathered on the increased genetic variation and pathogenicity of *P. infestans*, when new populations were observed in a number of geographical areas owing presumably to pathogen migration (Fry, 2008). Newly emerged populations of *P.*

infestans are often more aggressive compared to the old predominant populations, which is attributed to population displacement (Carlisle et al. 2002).

Currently the variability of the *P. infestans* population in terms of virulence in Ethiopia is unknown. Only anecdotal evidence suggests that some popular varieties, such as “Belete” show decreased levels of resistance in some areas of the country during some cropping seasons suggesting that different pathogen races may be present. Thus, it was necessary to study the races distribution and identification in the different potato growing regions of the country.

Material and Methods

The study area

The isolates of *P. infestans* were collected from major potato growing districts of Awi, West Gojam, and South Gondar Zones of Amhara region, northwestern Ethiopia in 2018 main cropping season staring from the end of June to end of August according to the occurrence of epidemic in the locations (Table 1). South Gonder is a dry high land, while Awi is wet high land and the majority of West Gojam is mid altitude and wet land area.

Table 1. Geographic locations of isolate collected zones of northwestern Ethiopia

Zone	Altitude (m)	Location latitude/ longitude	Rain fall (mm)	Temperature min/ max (°C)	Soil type	Soil Ph
Awi	2600	10.85 °N/36.8 °E	2300	8/22	Acrisol	4.8
South Gondar	2650	11.89 °N/38.04 °E	1500	11.8 / 23	Luvisol	5.2
West Gojam	2240	11.16 °N/37.29 °E	1211	11.57 / 26.89	Nitosol	

m.a.s.l= meter above sea level; mm= millimeter; °C= degree centigrade; min= minimum; max= maximum
Source: Baye et al., 2017

Sample collection and maintenance

Disease sample collections were done at 5-10 km interval along the road side. Sampling of *P. infestans* was carried out based on field symptom diagnosis. In each potato field, three leaves with single young lesion were collected and each leaf sample was put separately in plastic bags and brought to the laboratory.

Pathogen isolation

After taking the samples to the laboratory, 3 cm cuttings from healthy and the lesion were taken and surface sterilized with 10% commercial bleach solution for 30 seconds. The samples were then rinsed in sterile distilled water twice, dried off with sterile filter papers, and placed in a humid chamber in inverted Petri dish with water agar with the leaf’s abaxial side up. Plates were incubated at 18 °C for 5 to 6 days until fresh sporulation appears. Small pieces of infected tissue from the sporulating border of the lesion were cut and placed under surface sterilized Jalene—potato variety—tuber slices in an empty Petri dish. Surface sterilization was done by dipping potato tuber slices in 70% alcohol for 30 seconds, and burning off. Dishes were incubated at 18 °C for 6 - 7 days until there is abundant sporulation on the upper side of the slice. The mycelia

grown on the upper face of the slice were then transferred to healthy sterilized potato tuber slices for multiplication of each isolate (sample) for inoculum production. Microscopic checks have been done before transfer to potato tuber slices for further multiplication (Figure. 1). Inoculated potato tuber slices were then placed on sterilized wire netting in plastic boxes with damp filter paper at the bottom (CIP 2007). The boxes were closed in order to create a moist chamber, and incubate at 18 °C for 7 days (Figure 2).

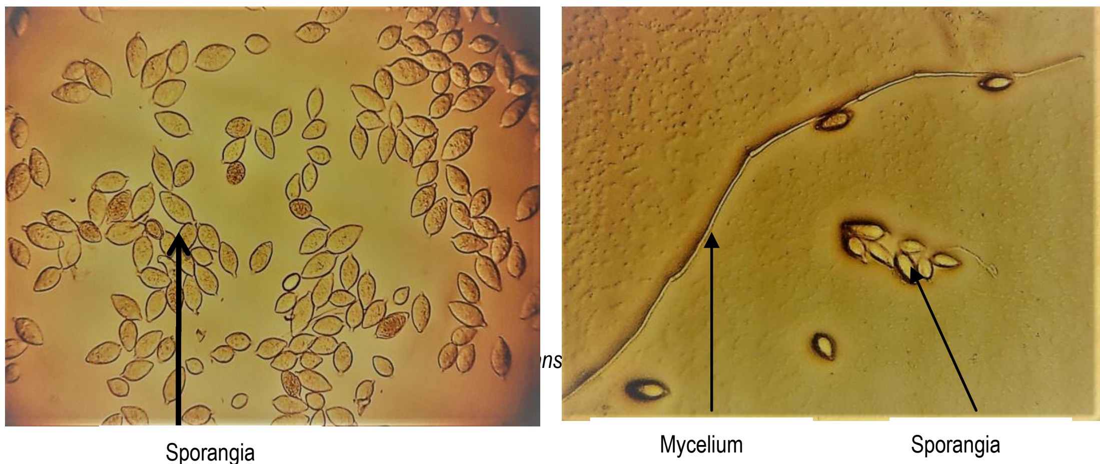


Figure 2. Multiplication of isolate on the tuber slices

Inoculating varieties and virulence determination

Plant material of differential varieties

The potato germplasm (differentials) consisting of 11 major R genes from R1 to R11 of *Solanum demissum* (Black et al. 1953) and one universal susceptible cultivar were obtained from the International Potato Center (CIP) gene bank (Table 2) and multiplication of plantlets was conducted at ARARI-TCL at Bahir Dar. The plant materials (i.e. *in vitro* plants) were cultured in the screen house covered /lined/ with plastic on the top. The potato differentials were planted at the interval of 15 days

staggered from mid-June to mid of July in sterile soil substrate in plastic pots of 23 cm diameter to synchronize the age of the differentials with occurrence of late blight in each zone i.e. to match collection of isolate samples from field and the age of the healthy leaves of the differentials to inoculate with the collected isolate samples. Leaves for the detached leaf assay were collected from 6 - 8 weeks old plants. Because the reactions of several R genes from *S. demissum* are reported to be sensitive to plant age and test conditions (Stewart, 1990), healthy and fresh leaves were selected from the middle part of the stem, while older, and senescent ones were avoided.

Table 2. Potato differentials containing the R genes from *Solanum demissum* that were used in the study

Clone	Accession name	Collecting number	CIP Accession number
Ma R0	r	Craigs Royal	CIP801038
1-Mar	R1	Craigs Snow White	CIP801039
2-Mar	R2	1512(16)	CIP801040
3-Mar	R3	Pentland Ace	CIP801041
4-Mar	R4	1563c(14)	CIP801042
5-Mar	R5	30053-18	CIP801043
6-Mar	R6	XD2-21	CIP801044
7-Mar	R7	2182ef(7)	CIP801045
8-Mar	R8	2424a(5)	CIP801046
9-Mar	R9	2573(2)	CIP801060
10-Mar	R10	3681ad(1)	CIP801047
11-Mar	R11	5008ab(6)	CIP801048

Determining virulence and sporulation intensity

For determination of virulence and sporulation intensity, 74 *P. infestans* isolates (Table 3) were used to inoculate a differential set of *S. demissum* carrying the major resistance R genes. Isolates are samples so here after when we say isolates it is to mean samples. Inocula of the isolates consisted of a sporangial suspension prepared from sporulated tuber slices adjusted (with a haemocytometer) to a concentration of 2×10^4 sporangia ml^{-1}. After 2 hours of conditioning at 4 °C to stimulate the release of zoospores from sporangia, droplets of sporangial suspensions were applied onto the abaxial side up of detached leaves collected from the differential cultivars and the universal susceptible cultivar on moist filter paper in a tray (60 × 25 cm). Trays covered by plastic bags were placed at 18 °C under a photoperiod of 16 hours light and 8 hours dark cycle during 7 - 8 days. For this experiment, three replicates were done for each isolate differential interaction and an isolate was considered to carry a virulence factor (VF) towards a specific R gene if the inoculation spots on the detached leaf of the differential cultivar showed visual necrosis. Virulence factors (VF) are those genes and proteins that play key roles in disease development.

Table 3. *Phytophthora infestans* isolates tested for virulence

Zone	District	Isolate assigned	Number of isolates
Awi	Guagusa Shikudad	A1-A10	10
	Banja	A11-A12	2
South Gondar	Lay Gayint	S1-S10	10
	Guna Begemidir	S11-S20	10
	Farta	S21-S32	12
West Gojam	Quarit	W1-W10	10
	Yilmana Densa	W11-W22	12
	Bahir Dar Zuria	W23-W30	8

A, S and W = Awi, South Gondar and West Gojam, respectively

Virulence of the isolates was evaluated in a binary fushion: sporulation present or absent (Figure 3). Avirulent isolates were those that did not sporulate and caused no lesions or lesions with clear edges, and not bigger than the area of the inoculation drop. Virulent isolates were able to sporulate and cause lesions of an area bigger than that of the inoculation drop.

Figure 3. Reaction of *P.infestans* isolates on detached leaves after 7 days of inoculation on R genes.

Virulence factors of each isolate were deduced from resistance and susceptibility patterns of each *Solanum* genotype. Numbers of virulence factors for each isolate were assessed from virulence patterns in order to deduce arbitrary classes called virulent, intermediate and avirulent. The isolate was ranged at an avirulent class when it has between 0 and 2 virulence factors. It was ranged at an intermediate class if it has 3 or 4 virulence factors. The isolate was classified at a virulent class when it has 5 virulence factors or more (Harbaoui et al. 2011).

Data analysis

The isolates were classified into races by integrating the virulence present at each differential. An isolate was considered to carry a virulence factor towards a particular R gene (differential cultivar) if it induced late blight symptoms. Race diversity was calculated as a standardized Shannon index (Andrivon 1994).

$$H = \sum_{i=1}^{S} -(P_i * \ln P_i)$$ - to make it normalized Shannon index (I), $I = H/\ln S$

Where:
H = the Shannon diversity index
P_i = fraction of the entire population made up of species i
S = numbers of species encountered
$\sum$ = sum from species 1 to species S

Race complexity of an isolate was determined according to the number of differential cultivars on which the isolate could induce late blight disease. A complexity index of "11" indicates that the isolate could infect all 11 differential cultivars while a complexity index of "0" indicates that the isolate was able to induce late blight disease on the universally susceptible cultivar only. All isolates carrying the same number of virulence factors were treated as having the same race complexity regardless of the combination of differential cultivars they showed virulence against. With 11 differential cultivars, there are 12 potential types (0–11) of race complexity.

Principal component analysis was computed to single out the R genes (differentials) that accounted the largest proportion to the total variation among isolates. Principal component analysis was done using XLSTAT. The scores of 74 isolates as "0" and "1" for absence and presence of infection on each R gene (differential), respectively, were used to calculate factor loads based on correlation matrix in principal component analysis to identify the relationships for much of the gross variability observed among isolates.

Results and Discussion

Virulence spectrum of *P. infestans*

Seventy-four *P. infestans* isolates; 12 from Awi, 32 from South Gondar, and 30 from West Gojam Zones were collected and used for race analysis study. The results of race identification based on reaction, revealed the occurrence of 6, 11 and 16 race types at Awi, South Gondar and West Gojam- respectively, in each population. Twenty-seven physiological races were identified in the pooled population when the 74 isolates from the three populations were combined (Table 4). Standardized Shannon index of physiological race diversity in the three populations were 0.75, 0.84 and 0.92 with a pooled Shannon index of 0.80 when the 74 isolates from the three populations were combined, which showed increased diversity of the pathogen population (Table 5). Among the 27 physiological races detected, 16 (59.26%) were observed only once.

The universal susceptible cultivar was infected by all isolates. The virulence factors on R9 and R5 genes were relatively rare only infected by 4 and 6 isolates, respectively, from 74 isolates tested. Almost all isolates were virulent on R1, R2, R3, R4, R6, R7, R10, and R11 differentials (Fig. 4). The northwestern race structure was highly complex, on average 6.3 virulence factors per isolate, but varied between locations.

Spectrum of pathogen race complexity was ranging from 0 to 10 virulence factors per isolate. Absence of virulence factors was detected in isolates S16 and W21 collected from South Gondar and West Gojam. The highly virulent factor (10 VF) meaning the highest number of defeated R genes was detected in three isolates (S10, S17, and S29) of the population from South Gondar (Table 4). Pathogen isolates collected from West Gojam exhibited the highest 9 virulence factors per isolate. Pathogen isolates collected from South Gondar and West Gojam grouped under avirulent, intermediate, and virulent categories though virulence factor fluctuate from the lowest to the highest virulent factor profiles. However, all isolates were in virulent class with lowest (6 VF) to the highest (9 VF) profiles in Awi zone. Generally, the most frequent category in the tested *P. infestans* population was 9 VF followed by 8 and 7 VF. Categories 0 and 4 VF was present with equal proportions in the population and each one comprised two isolates. The lowest frequency was detected for 5 VF categories (Table 4).

The most common virulence or more than 87% of isolates were virulent to R1, R2, R3, R4, R6, R7, R8, R10, and R11while 12% of isolates were virulent to R5 and R9. All isolates from Awi were virulent on R1, R4, R7, R8 and R11 differentials while the higher proportion of isolates from South Gondar were virulent to R2, R3, R6 and R10 genes. The isolates collected from West Gojam were virulent to all R genes except R9 and had lower proportion across differentials as compared to isolates collected from the other two zones. It was reported that isolates showed 100% of virulent forms against R1, R3, R4, R7, R10, and R11 and frequent presence of virulence against R6 and R8 differentials (Anna et al. 2016). The least frequent was virulence against R9 and R5. Other authors (Śliwka et al. 2006; Chmielarz et al. 2014) also reported this. Virulence factors against R9 in highly complex pathotypes were reported by (Andrivon (1994), Andrivon et al. (1994) and Lehtinen et al. (2008). We found higher proportion of unique races (observed only once) i.e. out of 27 races 16 (59.26%) more than half of the identified pathotypes being found only once. This finding is in agreement with the findings of Runno-Paurson et al (2009) in Estonia they reported a higher proportion of nearly half of the identified pathotypes were unique races (being found only once).

Table 4. Phenotypic diversity and races frequencies of *Phytophthora infestans* isolates

Race	Isolated codes in location			Number of isolates	Number of Virulence factor
	South Gondar	West Gojam	Awi		
0	S16	W21	-	2	0
3	-	W4, W11 W17, W18	-	4	1
4.6.10.11	-	W5	-	1	4
1.2.3.4	-	W14	-	1	
3.4.6.7.11	-	W20	-	1	5
1.4.6.7.10.11	-	W7, W9	-	2	6
3.6.7.8.10.11	-	W22	-	1	
2.3.4.6.10.11	-	W2	-	1	
1.2.4.6.10.11	S27	-	-	1	
1.4.7.8.10.11	-	-	A11	1	
1.2.3.6.7.10.11	S20	W1	-	2	7
1.2.3.4.6.10.11	S19, S23	W3, W8, W10, W13, W15	-	7	
1.2.3.4.6.7.11	-	W16	-	1	
1.3.4.6.7.8.10.11	-	-	A10	1	8
1.2.3.4.6.7.10.11	S1, S2, S12, S18, S31, S32	W12, W25, W27	-	9	
1.2.3.4.6.8.10.11	S3, S4, S28	-	-	3	
1.2.3.6.7.8.10.11	S8	-	-	1	
2.3.4.6.7.8.10.11	-	W19, W24	-	2	
1.2.3.4.6.7.8.11	-	W28	-	1	
1.2.3.4.6.7.8.10.11	S7,S9, S11, S13, S15, S21, S22, S24, S25, S26 ,S30	W6, W23, W29, W30	A1, A2, A3, A4, A5, A6, A9	22	9
1.2.3.4.6.7.9.10.11	S14	-	-	1	
1.2.4.6.7.8.9.10.11	-	-	A7	1	
1.2.3.4.6.7.8.9.11	-	-	A8	1	
1.2.3.4.7.8.9.10.11	-	-	A12	1	
1.2.3.4.5.6.7.10.11	S5, S6	-	-	2	
2.3.4.5.6.7.8.10.11	-	W26	-	1	
1.2.3.4.5.6.7.8.10.11	S10, S17, S29	-	-	3	10
Total number of isolates				74	
Total number of races				27	

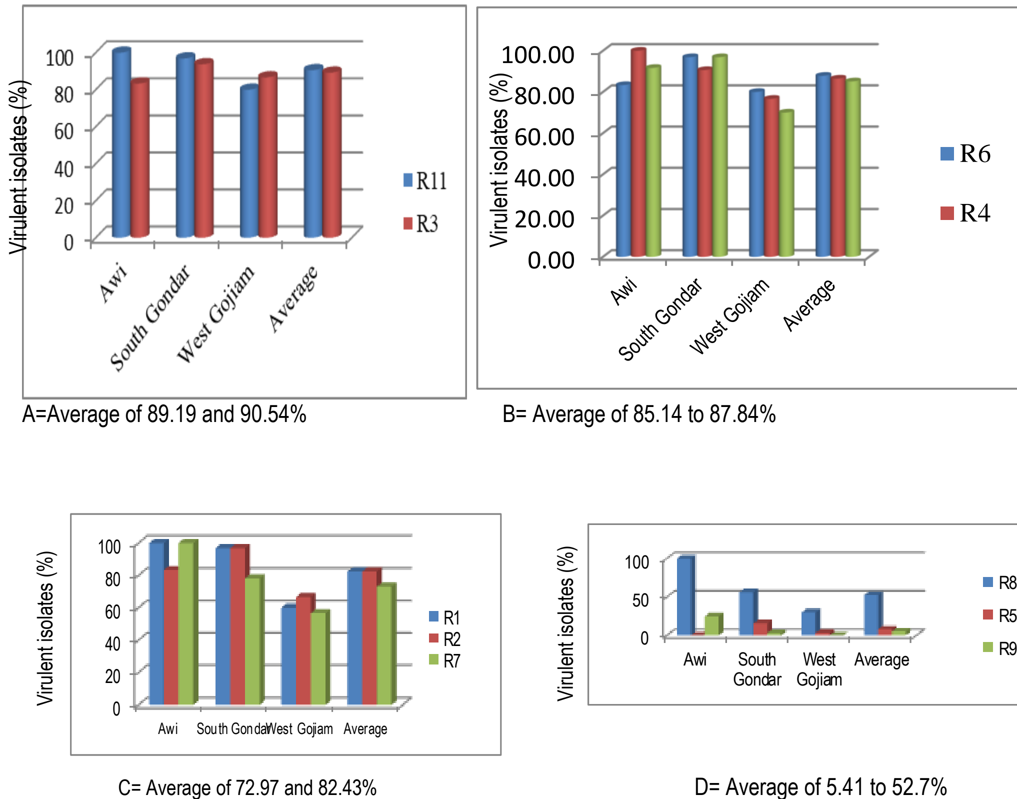
Table 5 Normalized Shannon diversity index of the physiological races

Population	Physiological race	
	Number	Normalized Shannon index
Awi Zone	6	0.75
South Gondar	11	0.84
West Gojam	16	0.92
Pooled	27	0.8

Distribution of virulent isolates on R genes

The virulent isolates on R11 and R3 were widely distributed in which 90.54 and 89.19% isolates from all zones were virulent on R11 and R3, respectively (Figure 4A) and 85.14 to 87.84% of isolates were virulent on R4, R6 and R10 (Figure 4B). Almost

73% of the isolates were virulent on R7 while 82.43% isolates were virulent on R1 and R2 (Figure 4C). More than half (52.7%) of the isolates were virulent on R8 while the lowest proportion of isolates (5.41%) was virulent on R9 (Figure D).



A=Average of 89.19 and 90.54%

B= Average of 85.14 to 87.84%

C= Average of 72.97 and 82.43%

D= Average of 5.41 to 52.7%

Figure 4A-D. Frequency of virulence of *Phytophthora infestans* isolates to potato R genes

All isolates collected from Awi Zone were virulent on R1, R4, R7, R8, and R11 while 96.88% of isolates collected from South Gondar were virulent on R1, R2, R6, R10 and R11 genes. A total of 3.13 and 25% of isolates from South Gondar and Awi zones, respectively, were virulent on R9, while 3.33 and 15.63% of isolates from West Gojam and South Gondar, respectively, were virulent on R5 gene.

Spatial distribution of virulent races of isolates across zones and districts

The largest number of isolates grouped under race 1.2.3.4.6.7.8.10.11 (22), 1.2.3.4.6.7.10.11 (9) and 1.2.3.4.6.10.11 (7) accounted for 51.35% of the isolates. The first race was detected from isolates collected from all zones and districts except from Banja and Yilmana Densa Districts of Awi and West Gojam, respectively. The other two races were identified from isolates collected from South Gondar and West Gojam, but these races were not identified from isolates collected from Quarit and Bahir Dar Zuria districts of West Gojam, respectively. Likewise, race 1.2.3.4.6.10.11was no

detected from isolates of Lay Gayint District of South Gondar. The three races were distinguished by incompatible reaction on R5 and R9, but race 1.2.3.4.6.10.11 differs from 1.2.3.4.6.7.8.10.11 because it was avirulent to R7 and R8 genes. Races 0 and 1.2.3.6.7.10.11 were identified from two isolates each; one from South Gondar and the other from West Gojam. Race 0 was known by consisting avirulent isolates to all R genes (R1 to R11) while race 1.2.3.6.7.10.11 was known by incompatible reaction on R4, R5, R8 and R9 genes (Table 6).

Race 3, 1.4.6.7.10.11 and 2.3.4.6.7.8.10.11 consisted of 4 (5.41%), 2 (2.7%) and 2 (2.7%) isolates, respectively, all collected from West Gojam. Race 3 was distinguished by incompatible reaction on all R genes except R3; race 1.4.6.7.10.11 was characterized by incompatible reaction on R2, 3, 5, 8 and 9 while race 2.3.4.6.7.8.10.11 was known by incompatible reaction on R1, 5 and 9 genes. Race 1.2.3.4.6.8.10.11 and 1.2.3.4.5.6.7.8.10.11 were detected from three isolates each. Each three isolates and race 1.2.3.4.5.6.7.10.11 consisted of two isolates all collected from South Gondar. Race 1.2.3.4.6.8.10.11 was distinguished by incompatibility on R5, R7, and R9 while race 1.2.3.4.5.6.7.8.10.11 was characterized by incompatible reaction on R9. Race 1.2.3.4.5.6.7.10.11 was distinguished by being virulent on R5 but avirulent on R8 and R9 genes. The other 16 races were detected only from one isolate each (Table. 6).

The results indicated that the migration of virulence races across zones. This indicated that it is more likely that adjacent zones might experience similar virulence races. However, the dissimilarity of races detected from some districts of same zones might be due to the less exchange of planting materials within districts of zones or the unfavorable environments for some races in particular district(s) of same zone. According to Fry (2008) migration events have contributed in shaping the population structure of *P. infestans* in a number of locations around the world. The races of *P. infestans* can be dispersed anywhere in the country as it cannot restricted to one region (Drenth et al. 1995; Goodwin and Sujkowski 1995). The observed variations among and within samples of *P. infestans* collected from eight districts of three zones is in agreement with other report that suggested the genetic variation and pathogenicity of *P. infestans* increased when new populations are observed in a number of geographical areas owing presumably to pathogen migration (Fry 2008).

The results of this study showed differences in spatial distribution of virulence factors and race structure of the pathogen. The isolate samples collected from West Gojam were most diversified in which 53.33% of isolate samples grouped into 11 separate physiological races while 41.67% of isolates collected from Awi and 34.38% isolates collected from South Gondar were grouped into 5 and 6 separate physiological races, respectively, without including isolates from other zones (Table 4 and 6). The difference in spatial distribution of virulence factors and race structure complexity of the pathogen among samples of three populations might be due to variations of weather variables such as temperature ranges within the zones or the growing of different cultivars localized in different areas. Singh and Pundhir (2011) suggested the

introduction of increasing number of new potato varieties might have resulted in the occurrence of a good number of races of the *P.infestans*.

Table 6. The spatial distribution of virulent races of *P. infestans* isolates

Spatial distribution of virulent isolates					
Zone	**District**	**Race**	**Isolate code**	**Number of isolate**	**Share in %**
Awi	Guagusa Shikudad	1.2.3.4.6.7.8.10.11	A1, A2, A3, A4, A5, A6, A9	7	9.46
South Gondar	Lay Gaint		S7, S9,	2	2.7
	Guna Begemidir		S11, S13, S15	3	4.05
	Farta		S21, S22, S24, S25, S26, S30	6	8.11
West Gojam	Quarit		W6	1	1.35
	Bahir Dar Zuria		W23, W29, W30	3	4.05
Awi	Guagusa Shikudad	1.2.4.6.7.8.9.10.11	A7	1	1.35
	Guagusa Shikudad	1.2.3.4.6.7.8.9.11	A8	1	1.35
	Guagusa Shikudad	1.3.4.6.7.8.10.11	A10	1	1.35
	Banja	1.4.7.8.10.11	A11	1	1.35
	Banja	1.2.3.4.7.8.9.10.11	A12	1	1.35
South Gondar	Lay Gaint	1.2.3.4.6.7.10.11	S1, S2	2	2.7
	Guna Begemidir		S12, S18,	2	2.7
	Farta		S31, S32	2	2.7
West Gojam	Yilmana Densa		W12	1	1.35
	Bahir Dar Zuria		W25, P27	2	2.7
South Gondar	Lay Gaint	1.2.3.4.6.8.10.11	S3, S4	2	2.7
	Farta		S28	1	1.35
South Gondar	Lay Gaint	1.2.3.4.5.6.7.10.11	S5, S6	2	2.7
	Lay Gaint	1.2.3.6.7.8.10.11	S8	1	1.35
South Gondar	Lay Gaint	1.2.3.4.5.6.7.8.10.11	S10	1	1.35
	Guna Begemidir		S17	1	1.35
	Farta		S29	1	1.35
South Gondar	Guna Begemidir	1.2.3.4.6.7.9.10.11	S14	1	1.35
South Gondar	Guna Begemidir	0	S16	1	1.35
West Gojam	Yilmana Densa		W21	1	1.35
South Gondar	Guna Begemidir	1.2.3.4.6.10.11	S19	1	1.35
	Farta		S23	1	1.35
West Gojam	Quarit		W3, W8, W10	3	4.05
	Yilmana Densa		W13, W15	2	2.7
South Gondar	Guna Begemidir	1.2.3.6.7.10.11	S20	1	1.35
West Gojam	Quarit		W1	1	1.35
South Gondar	Farta	1.2.4.6.10.11	S27	1	1.35
West Gojam	Quarit	2.3.4.6.10.11	W2	1	1.35
West Gojam	Quarit	3	W4	1	1.35
	Yilmana Densa		W11, W17, W18	3	4.05
West Gojam	Quarit	4.6.10.11	W5	1	1.35
	Quarit	1.4.6.7.10.11	W7, W9	2	2.7
	Yilmana Densa	1.2.3.4	W14	1	1.35
	Yilmana Densa	1.2.3.4.6.7.11	W16	1	1.35
West Gojam	Yilmana Densa	2.3.4.6.7.8.10.11	W19	1	1.35
	Bahir Dar Zuria		W24	1	1.35
West Gojam	Yilmana Densa	3.4.6.7.11	W20	1	1.35
	Yilmana Densa	3.6.7.8.10.11	W22	1	1.35
	Bahir Dar Zuria	2.3.4.5.6.7.8.10.11	W26	1	1.35
	Bahir Dar Zuria	1.2.3.4.6.7.8.11	W28	1	1.35
Total				**74**	**100**

A, S and W = Awi, South Gondar and West Gojam, respectively.

Air temperature might be representing a major driver of the evolution of virulence factors and race complexity in *P. infestans* populations in the study areas. Dell et al. (2011) indicated that temperature is one of the most important environmental parameters that can have crucial impacts on nearly all aspects of biological processes. Shapiro and Cowen (2012) identified that in host-pathogen interactions, temperature can have not only a critical influence on the occurrence and severity of disease epidemics in the short-term but also on the longer-term evolutionary trajectory of pathogens. E-Jiao (*et al.* 2016) strengthen the hypothesis of temperature-mediated dynamics of virulence factors and race structure in *P. infestans* by significant associations of virulence frequency and race complexity in pathogen populations with the mean annual temperature in the sites where pathogen populations were sampled in China.

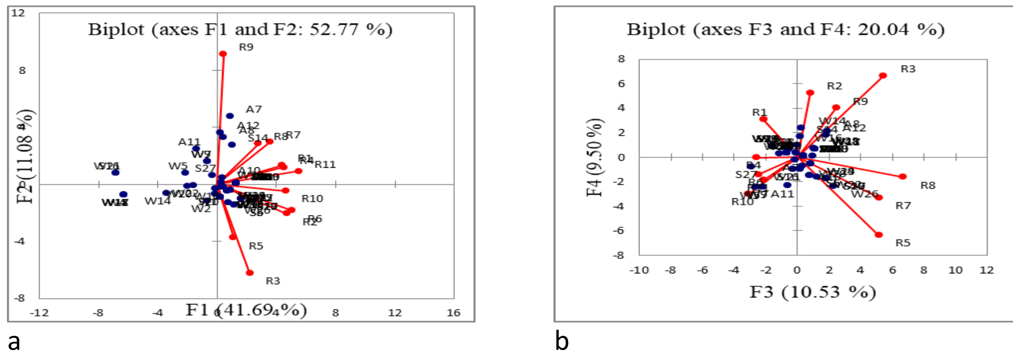
Principal component analysis

The first four factors contributed to 72.08% to the total variations of isolates in which factor 1 (F1) and 2 (F2) contributed 41.693 and 11.076%, respectively, with cumulative contribution of 52.77%. The larger contribution of F1 was due to the cumulative contribution of R1, R2, R3, R6, R7, R10, and R11 in the range between 7.7 and 18.24%, while R3 and R9 had larger contribution of 23.49 and 50.5%, respectively, to F2 factor load. R8 contributed 27.07% to F3, and R5 and R3 had larger contribution of 24.43 and 26.81%, respectively, to F4 factor load (Table 7). The larger contribution of these differentials to the total variability of isolates was visualized by the longer rays of R9 and R3 genes (Figure 5A) and R3, R5 and R8 (Figure 5B) of biplots of differentials and isolates. The isolates, A7, A8 and A12 collected from Guagusa Shikudad and Banja and S14 isolate collected from Guna Begemidir were virulent on R9 differential (Figure 5A). The isolate, A10 collected from Guagusa Shikudad, S6 and S20 isolates collected from Lay Gayint and Guna Begemidir and W20, W22 and W26 isolates collected from Yilmana Densa and Bahir Dar Zuria were virulent on R5 differential (Figure 5B).

Table 7. Factors load and contribution of 11 R genes used as differentials to variation of 74 isolates of *P. infestans*

Differential	F1	F2	F3	F4
R1	0.727 (11.52%)	0.114 (1.07%)	-0.180 (2.8%)	0.244 (5.71%)
R2	0.780 (13.28%)	-0.177 (2.56%)	0.069 (0.41%)	0.416 (16.59%)
R3	0.365 (2.91%)	-0.535 (23.49%)	0.457 (17.99%)	0.529 (26.81%)
R4	0.749 (12.24%)	0.103 (0.87%)	-0.216 (4.04%)	-0.001 (0.0%)
R5	0.183 (0.73)	-0.321 (8.46%)	0.430 (15.96%)	-0.505 (24.43%)
R6	0.842 (15.44%)	-0.158 (2.04%)	-0.205 (3.61%)	-0.110 (1.16%)
R7	0.594 (7.70%)	0.257 (5.43%)	0.431 (16.04%)	-0.261 (6.51%)
R8	0.460(4.62%)	0.245 (4.93%)	0.560 (27.07%)	-0.129 (9.75%)
R9	0.0721 (0.11%)	0.784 (50.5%)	0.205 (3.63%)	0.319 (9.75%)
R10	0.778 (13.21%)	-0.040 (0.13%)	-0.258 (5.76%)	-0.238 (5.43%)
R11	0.915 (18.24%)	0.080 (0.53%)	-0.177 (2.69%)	-0.146 (2.04%)
Eigen value	4.586	1.218	1.159	1.045
Variability (%)	41.693	11.076	10.534	9.501
Cumulative %	41.693	52.770	63.304	72.805

Numbers in parenthesis indicate percent contribution of each differential (R-gene) to each factor load



a b

Figure 5a and b. Biplot of differentials (R1-R11 genes) and isolates on F1 and F2 as well as F3 and F4 axes showing the contribution of R genes to total variations of sample isolates

The results from principal component analysis showed that R3, R5, R8, and R9 had the larger contribution to the total variability of sample populations of *P. infestans.* Moreover, majority of sample isolates (86.49%) grouped under 20 races of isolates were avirulent to R5 and R9 genes and 13.51% isolates collected from six districts of the three zones were virulent to R5 and R9 genes. This might suggest the importance of using these resistant genes for gene stacking in the development of resistant varieties for the management of late blight disease in the districts where isolates exhibited avirulence on R5 and R9 differentials. Therefore, effective management of the disease in the region need to be directed in development of varieties for none race-specific (horizontal) resistance. The race-specific resistance in potato to *P. infestans*, conferred by single dominant R-genes is short-lived because of rapidly changing pathogen populations (Vanderplank 1971). This fact was reported by many authors of which potato cv. Pentland Dell was released in 1961 in the United Kingdom which possessed genes R1, R2, R3, and R4, but resistance was soon broken by new virulent race R4 (compatible with R4). Race-specific resistance is controlled by R genes leads to a race-specific hypersensitive response and provides only short time resistance in the field because the resistance is overcome with the appearance of new virulent races of the pathogen (Song *et al.* 2003).

Conclusion

Phytophthora infestans populations in northwestern Ethiopia were complex and characterized by high virulence race diversity. All the R genes were overcome by races collected from different growing areas of the region and only R9 and R5 genes seems effective which withstood about 95% and 92% of the isolates tested, respectively. The use of race-specific resistance genes is considered as effective for managing late blight. However, it is hardly possible to suggest that breeding for major gene resistance is effective to control late blight disease in the study area due to the presence of virulent genes of the pathogen for all R genes in all zones and the migration and fast distribution of the races anywhere in the growing areas. Therefore, it is suggested to use integrated management of the disease depending on a year-to-year study results on

the change taken place in the populations of the pathogen until the potato varieties for horizontal resistance are developed.

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