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Population structure of *Gibberella xylarioides* Heim and Saccas in Ethiopian forest coffee (*Coffea arabica* L.) systems

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Coffee wilt caused by *Gibberella xylarioides* (*Fusarium xylarioides*) is a troublesome soil borne disease of *Arabica* coffee (*Coffea arabica L.*) in Ethiopia. It has been known to be prevalent and severe in plantation, garden and semi-forest coffee production systems in that order of importance. A number of recent reports have also indicated that the disease is equally damaging coffee trees with varying intensities thereby endangering the conservation of wild coffee genetic resources in forest coffee systems of the country. However, the reasons for the disease upsurge in the forest remain speculative. Thus, population structure of coffee wilt pathogen was studied by cross inoculating 12 accessions with four isolates collected in the four forest sites, namely, Bonga, Berhane-Kontir and Yayu (southwest) and Harenna (southeast) of Ethiopia. A pathogenic isolate 'Gx11' and a moderately resistant coffee cultivar *cv*. 7440, both from plantation were included as respective standard checks. The cultural and morphological characteristics of 24 isolates from the forests were compared with six strains collected from semi-forest and plantation coffee. The cultural appearance of most isolates from southwest was generally similar in pigments, aerial and radial growths but relatively different from those isolates collected in the Southeast forest site (Harenna). The result of coffee accession by isolate interactions showed that accessions of Harenna (P4, P6 and P11) were resistant to almost all isolates (except to its isolate) with low mean percent seedling deaths (< 31%) while Bonga (P27) and Berhane-Kontir accessions (P34 and P38) were highly susceptible to all isolates with higher seedling deaths of 79.2 to 85.7%. The Harenna isolate was most aggressive (78.7%) followed by Bonga 'B23' and Yayu 'Y21' isolates which were as aggressive as the one from plantation coffee 'G11'. In conclusion, the fungus population structure in the forest coffee sites have basically similar cultural and morphological characteristics of the species *G. xylarioides* (*F. xylarioides*) with certain differences between southwest and southeast in colony growth nature, pigmentation and aggressiveness. The study evidenced that the pathogen strains in the forest coffee are equally or even more aggressive than those strains in other coffee production systems, thus rapidly threatening *Arabica* coffee gene pool of Ethiopia.

Key words: Aggressiveness, colony growth, forest coffee, *Fusarium xylarioides*, coffee production systems, host-pathogen interactions.

INTRODUCTION

Coffee wilt (*Gibberella xylarioides* Heim and Saccas) is a vascular troublesome soil-borne disease of the two commercially important coffee species namely, *Coffea arabica* L. in Ethiopia and *C. canephora* in Democratic

Republic of Congo (DRC), Uganda and Tanzania. This disease could be a great concern for future sustainability of coffee production in Eastern and Central Africa and of major potential threat to the world coffee like that of the historical coffee leaf rust and coffee berry disease (Rutherford, 2006; Flood, 2009; Girma et al., 2009a). In Ethiopia, it is one of the major biotic factors constraining the crop production with rapid prevalence and severity in plantation, garden and semi-forest coffee systems (Girma et al., 2001, 2009a; Girma, 2004; Musebe et al., 2009). Recently, Sihen et al. (2012) reported that coffee wilt is causing significant losses to coffee trees in the forest coffee systems of Ethiopia inhabiting invaluable gene pools of *C. arabica*. The highest mean incidence was about 29.2% in Harenna forest coffee (southeast), followed by Brehane-Kontir (southwest) with mean incidences that varied from 22 to 28% during 2008 and 2009 seasons (Sihen et al., 2012). Zeru et al. (2009) earlier reported that the coffee tree losses ranged from 2.4% in Berhane-Kontir to 17% in Yayu forest areas in 2005. The overall comparison of coffee wilt progress over the years indicated that the disease pressure has been increasing in the forest coffee across all sites demonstrating its remarkable importance as is in garden and plantation coffee systems (Sihen et al., 2012).

Among the reasons for coffee wilt upsurge could be due to dynamic shift in the pathogen and/or host populations over space-time (spatio-temporal dynamics) that might have been imposed by the diversity of coffee types (wild, landrace, varieties) selection pressure and/or change in composition and/or buildup of aggressive strains of the fungus beside the other factors. Sihen et al. (2012) hypothesized that the inconsistent response of Harenna coffee accessions under natural infections and the artificial inoculation tests (greenhouse conditions) gives clue to the differences in aggressiveness between the fungus populations and/or variation in the host reactions. Burdon (1993) argued that the population biology of pathogens in agricultural and natural communities is most likely different, attributing to ecological constraints and opportunities imposed by agricultural and natural plant communities with considerable effects on the basic principles of population dynamics. The resultant change in the relative importance of particular phenomena often produces markedly different epidemiological and genetical consequences for pathogen populations in these different environments (Burdon, 1993). In addition, Brown and Tellier (2011) recently pointed out that there is selection pressure on plants for resistance to parasites and equally on parasites to overcome host defenses. This confrontation drives coevolution, in which gene frequencies in one species determine the fitness of genotypes of the other species, and leads to diversity in host defenses and parasite weaponry (Brown and Tellier, 2011). With regard to coffee wilt, a number of authors (Adugna et al., 2005; Girma et al., 2009b; Musoli et al. 2009) documented that there are variations in *Coffea-Gibberella xylarioides* pathosystem. These results, however, are largely emanated from studies conducted on coffee varieties or lines and the fungus isolates originated from semi-forest and plantation production systems. So far, there is little equivalent knowledge concerning the host-pathogen interactions in 'wild' forest coffee systems. Thus, the main objectives of this study were to examine the population structure of *G. xylarioides* (*Fusarium xylarioides*) isolates and coffee accessions originated from four contrasting 'wild' forest coffee sites in comparison with known strains isolated from semi-forest and plantation coffee in Ethiopia.

MATERIALS AND METHODS

Collections of fungal isolates

Pure cultures of 24 *G. xylarioides* (*F. xylarioides*) isolates representing four forest coffee sites were selected from large collections in different plots of Bonga, Berhane-Kontir and Yayu (southwest) and Harenna (southeast) of Ethiopia. Then, the isolates were compared with six known strains of the pathogen obtained from semi-forest coffee at Jimma and that of plantation at Gera in southwest Ethiopia (Table 1). The isolates were first retrieved on fresh Spezieller Nährstoffarmer agar (SNA) (Nirenberg, 1976) medium, from the stock culture on slant agar maintained at 4°C, and grown for a week and then used for cultural and morphological characterization. Out of these, four fungal isolates (each per locality) and a strain from Gera were selected based on colony growth to study host-pathogen interaction.

Cultural characterization of the isolates

For cultural characterization, each isolate was transferred from SNA onto potato sucrose agar in 90 cm Petri dishes and, grown under dark for the first three days, and then exposed to fluorescent light/dark cycle at 22 ± 1°C temperature (Booth, 1971; Rutherford et al., 2009). Cultural appearances such as colony color (upper and lower), and aerial as well as radial growth were scored for 21 days incubation at every three to five days interval. The apical mycelia growth was rated visually as appressed (flat), slightly raised and raised from the agar surface; and the colony density of isolates was recorded as dense, intermediate and sparse (Girma, 2004). Radial growth was measured with transparent ruler (mm), as colony diameter from two perpendicular planes on the reverse side of the Petri dishes. Colony color on obverse (upper) and types of pigments from the reverse (under side) of the Petri dish for each isolate was determined using color chart.

Morphological characterization of the isolates

All the isolates were grown on SNA plates for studying microscopic structures of the fungus under aforementioned growth conditions (Booth, 1971; Gerlach and Nirenberg, 1982; Rutherford et al., 2009). Fourteen-day old cultures of each isolate was flooded with 10 ml distilled sterile water and rubbed gently from the agar surface to free the fungus conidia. The spore suspensions were filtered through cheese clothes into sterile beaker, while thoroughly stirring,

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Table 1. Frequency of aerial mycelial growth and colony density of *Fusarium xylarioides* (*Gibberella xylarioides*) isolates collected in southeast and southwest forest coffee sites of Ethiopia and incubated on potato sucrose agar under 12 h light/dark cycle at 22 ± 1°C.

Forest coffee site	Aerial growth of mycelia (%)			Mycelia density (%)	
	Slightly raised	Raised	Appressed	Slightly dense	Dense
Harenna (n= 6)[1]	66.7	33.3	0	83.3	16.7
Bonga (n= 5)[1]	80	0	20	100	0
Berhane-Kontir (n= 5)[1]	80	0	20	100	0
Yayu (n= 8)[1]	80	0	20	88.9	11.1
Jimma (n= 3)[2]	100	0	0	100	0
Gera (n= 3)[3]	100	0	0	100	0
Mean and SD	84.5 ± 13.1	5.6 ± 13.6	10 ± 10.9	95.4 ± 7.4	4.6 ± 7.4

n = number of isolates from each locations, [1]forest coffee, [2]semi-forest and [3]plantation coffee. SD = standard deviations.

and then slides were prepared for microscopic examination. The types of conidia as micro- and macro- were identified based on the size and the number of septa, and the shape of apical and basal cells, and their respective frequencies were tallied with the microscope. The conidial size (length and width) was measured with calibrated ocular micrometer (µm) fitted into 10x eyepiece and 40x objective. The presence and/or absence of fruiting bodies like chlamydospores and/or perithecia arising in the culture were also checked at older stage (Booth, 1971; Summerell et al., 2003; Girma, 2004; Rutherford et al., 2009).

Host-pathogen interaction study

The host-pathogen interaction was studied in the greenhouse at Jimma through cross inoculation of 12 *Arabica* coffee accessions with four *G*. *xylarioides* isolates originated from the four wild forest sites, namely; Bonga, Berhane-Kontir, and Yayu (southwest) and Harenna (southeast) in Ethiopia during the period of 2009 and 2010. Also, a coffee variety with moderate resistance to coffee wilt *cv* '7440' and a reference strain of the fungus 'G11' collected from plantation at Gera were used as standard checks. Seeds were prepared from the representative coffee accessions (three per locality), originally collected from each forest coffee site and then conserved at Jimma Agricultural Research Center (JARC) (Sihen et al., 2012). Seedlings were raised by sowing the coffee seeds in sterilized and moistened sandy soil in plastic pot of 5.8 L volume (20 to 25 seeds/pot and three pots/accession) after removing the parchment and soaking overnight (Girma et al., 2009b; Sihen et al., 2012). At cotyledon stage of coffee seedlings, inoculum of each of the four *G. xylarioides* isolates was multiplied and independently inoculated to each seedlings (20 per pot) of 13 accessions (12 accessions and a check) at 2.1 × 10^6 conidia/ml concentration determined by haemocytometer following the stem nicking technique described in detail by Girma et al. (2009b) and Sihen et al. (2012). As a negative control, five seedlings of *cv*. 7440 were treated with distilled sterile water by the same procedure.

The inoculated plants were immediately placed in air-conditioned growth room with temperature of 23 ± 1°C and fluorescent light, and covered with misted transparent plastic sheet to maintain higher humidity to ensure disease infection. After a week, the seedlings were transferred and placed on experimental benches in the greenhouse for disease assessment.

Experimental design, data collection and statistical analysis

The laboratory study was conducted in a Completely Randomized Design (CRD) replicated three times, and the pathological analysis was laid out in randomized complete block design (RCBD) in a factorial treatment combinations of five isolates by thirteen accessions replicated three times using 20 seedlings per pot. Qualitative parameters such as apical growth, colony density, colony color and pigment and spore shapes were visually scored. Quantitative characters like radial growth (mm) and conidial size (µm) were measured and recorded on 25 to 30 spore samples. The first date wilting seedling observed and the number of wilted/dead seedlings per pot was recorded at fourteen days interval for six months after inoculation. Finally, the incubation period (days) and percentage of dead seedlings were computed (Girma et al., 2009b; Sihen et al., 2012). The respective data sets were statistically analyzed with SAS 9.2 version (SAS Institute, 2008).

RESULTS AND DISCUSSION

Cultural characteristics of *Gibberella xylarioides*

The colony color was generally grayish white (beige) when observed from the upper side, but different pigments that varied from grayish (with or without bluish spots) and purplish white to light violet and light bluish were observed on the reverse side of the plates. These characteristic pigments were produced on potato sucrose agar when incubated under 12 h light/dark cycles at 22 ± 1°C for 10 to 14 days. The observed variations broadly correspond with origin of the isolates and almost all the Harenna group were grayish white (85%), while Bonga, Berhane-Kontir and Yayu isolates had grayish white (40%), light bluish (25%), light purplish (20%) and violate (15%) on reverse side of the plate.

The cultures of 30 *G*. *xylarioides* isolates have invariably shown typical growth, color and pigment descriptions of anamorph state *F*. *xylarioides* (Booth, 1971; Girma, 2004; Rutherford et al., 2009). Based on the aerial mycelia growth, the isolates were grouped as appressed (flat)-sparse, slightly raised-slightly dense (intermediate) and raised and dense texture. In this case also, the Harenna isolates differed from the others with slightly raised and slightly dense (66.7%) and flat-sparse (33.3%) colony as opposed to Bonga, Berhane-Kontir

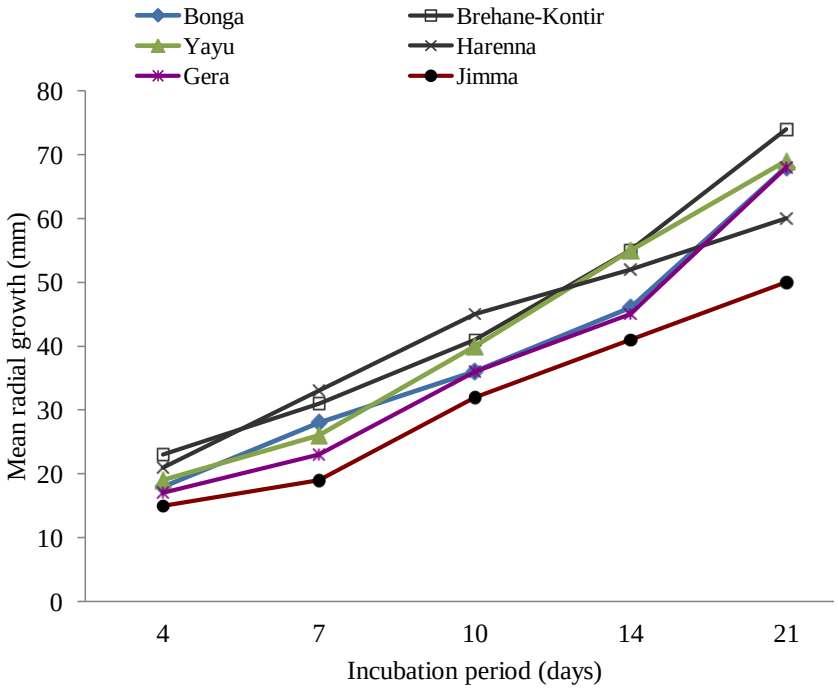

Figure 1. Mean radial colony growth rate (mm/day) of *Fusarium xylarioides* (*Gibberella xylarioides*) isolates collected from forest coffee sites of southwest and southeast Ethiopia when grown on potato sucrose agar at 22 ± 1°C and 12 h light/dark cycles.

and Yayu populations that showed intermediate growth type slightly raised-dense (80%) and 20% was appressed (flat) and sparse. Similarly, the six isolates from Gera and Jimma were also intermediate types (Table 1). There was significant ($P < 0.05$) difference in radial colony growth among *G. xylarioides* isolates across the forest coffee sites. The mean colony growth of Bonga isolates were 16.3, 23.8, 32.6, 42.9 and 63.4 mm; whereas 23.1, 31.4, 42.2, 56.1 and 71.1 mm was recorded for Berhane-Kontir population at 4, 7, 10, 14 and 21 days of incubation periods.

The radial growth rate analysis showed that the Harenna isolates grow faster than those isolates from southwest coffee areas including Gera and Jimma during the first 10 days of incubation; although, they became slower than the other groups as colonies got older. Berhane-Kontir isolates exhibited the reverse growth pattern (Figure 1). Similar to the present study, Girma (2004) reported varying radial growth rate among *G. xylarioides* populations collected from different coffee types and geographic localities in Africa. Among the *Arabica* isolates, those from the southern region relatively grow at slower rates (3.1 mm/day) than those isolates collected in southwestern (4.0 mm/day) Ethiopia. The *Robusta* and *Excelsa* strains of the fungus had 3.6 and 3.4 mm/day, respectively, grown on the same medium under similar conditions (Girma, 2004). In general, the cultural characteristics of *Arabica* isolates ranged from very sparse and appressed to dense and raised colony with grayish, light bluish and purplish or violate pigments as opposed to the strains from *Robusta* and *Excelsa* coffee which were orange, flat and pionnotal in appearance (Booth, 1971; Girma, 2004; Rutherford et al., 2009).

Microscopic appearances of *Gibberella xylarioides*

The *Fusarium* state of *G. xylarioides* (*F. xylarioides*) produces macroconidia and microcondia variable in shape and size with 1 to 3 septate. The macroconidia are cylindrical, slightly curved and curved with hooked ends while the microconidia are frequently allantoid, comma- and U-shaped with or without a septum. The average macroconidia size ranged from 17.6 × 2.8 to 25.0 × 2.9 µm, while that of microcondia varied between 10.9 × 2.9 and 11.8 × 2.9 µm (Table 2). The findings on the cultural and morphological characteristics of this fungus are in agreement with the reports of previous work on large number of strains collected from *Arabica* and *Robusta* coffee (Gerlach and Nirenberg, 1982; Girma, 2004; Rutherford et al., 2009).

Coffee accessions by *Gibberella xylarioides* isolates interactions

The results of host-pathogen interactions studied by inoculating seedlings of 13 forest coffee accessions with

Table 2. Shape and size of conidia of *Fusarium xylarioides* (*Gibberella xylarioides*) isolates collected from four forest coffee sites in southeast and southwest Ethiopia and grown on SNA at 22 ± 1°C and 12 h light/dark cycle for 14 days.

Location	Shape and size (L × W) (µm)				
	Microconidia		Macroconidia		
	Allantoid[1]	U-shaped[1]	Cylindrical[2]	Curved[2]	Slightly curved[2]
Harenna	10.9 × 3.1	9.6 × 2.9	20.8 × 2.8	22.3 × 2.8	24.1 × 2.9
Bonga	12.1 × 2.9	11.2 × 2.8	24.9 × 2.9	21.4 × 2.9	25.7 × 2.9
Berhane-Kontir	12.5 × 2.9	12.1 × 2.9	21.4 × 2.7	20.7 × 2.9	27.5 × 3.0
Yayu	13 × 2.9	13.4 × 2.9	25.2 × 2.9	20.1 × 2.8	25.4 × 2.9
Jimma	10 × 3.1	9.6 × 2.9	20.8 × 2.8	22.3 × 2.8	24.1 × 2.9
Gera	12.5 × 2.5	9.6 × 2.9	21.9 × 2.7	18.6 × 2.7	23 × 2.7
Mean	11.8 × 2.9	10.9 × 2.9	22.5 × 2.8	17.6 × 2.8	25.0 × 2.9

[1, 2]refers to the shape and size of micro- and macro-conidia.

Table 3. Coffee wilt severity (mean percent seedling death[1]) on twelve coffee accessions inoculated with four *Gibberella xylarioides* isolates collected from the forest sites of southwest and southeast Ethiopia and grown under greenhouse condition at Jimma in 2010.

Forest coffee accession[2]	*Gibberella xylarioides* isolate[3]					
	B23	SH21	Y21	H11	G11	Mean
P4	10.0^{jk}	0.0^{3k}	0.0^{k}	90.0^{a}	9.6^{jk}	21.9^{G}
P6	13.2^{jk}	0.0^{k}	4.1^{k}	77.7^{a-e}	12.4^{jk}	21.5^{G}
P11	30.4^{ij}	0.0^{k}	31.3^{ij}	78.8^{a-e}	27.7^{ij}	33.6^{F}
P17	44.2^{g-i}	13.9^{jk}	16.5^{jk}	55.9^{e-h}	48.1^{f-i}	35.7^{EF}
P21	45.1^{g-i}	63.3^{b-h}	66.3^{b-g}	56.8^{d-h}	47.3^{f-i}	55.8^{D}
P27	82.1^{a-c}	64.9^{b-h}	79.3^{a-d}	90.0^{a}	79.4^{a-d}	79.2^{AB}
P34	90.0^{a}	42.7^{hi}	69.9^{a-f}	90.0^{a}	90.0^{a}	76.5^{AB}
P38	90.0^{a}	76.5^{a-e}	86.2^{ab}	85.8^{a-c}	90.0^{a}	85.7^{A}
P41	90.0^{a}	9.5^{jk}	90.0^{a}	90.0^{a}	84.3^{a-c}	72.8^{B}
P47	90.0^{a}	0.0^{k}	90.00^{a}	90.0^{a}	80.6^{a-c}	70.1^{BC}
P49	86.2^{ab}	0.0^{k}	90.0^{a}	86.0^{a-c}	90.0^{a}	70.4^{BC}
P59	85.5^{a-c}	0.0^{k}	90.0^{a}	63.0^{c-h}	69.1^{a-f}	61.5^{CD}
7440	67.0^{a-g}	0.0^{k}	18.8^{jk}	68.5^{a-f}	71.5^{a-f}	45.2^{E}
Mean	63.4^{X}	20.8^{Z}	56.4^{Y}	78.7^{W}	61.5^{XY}	

[1]Percent wilt was calculated from cumulative number of dead over total number of seedlings (20 per treatment) six months after inoculation, and the actual wilt values were arcsine-square root transformed to normalize the data; [2]Coffee accessions P4, P6 and P11 selected from Harenna; P17, P21 and P27 from Bonga; P34, P38 and P41 from Berhane-Kontir; and P47, P49 and P59 from Yayu forests; and 7440 moderately resistant coffee variety; [3]*Gibberella xylarioides* isolates coded as B23, SH21, Y21 and H11 selected from Bonga, Berhane-Kontir, Yayu and Harenna forest coffee sites, respectively; and isolate G11 is collected from Gera coffee plantation; [3]0.0 (zero value) indicates there was no seedling death during the study period (six months after inoculation). Means followed with the same letter(s) are not significantly ($P < 0.05$) different from each other according to Duncun's Multiple Range Test (DMRT). LSD values for the coffee accessions, the isolates and the interactions comparison were 10.3, 6.4 and 23.1, respectively. Coefficient of variations (CV) = 25.41%.

five *G. xylarioides* isolates (including checks) showed significant ($P < 0.01$) main (accessions and isolates) and interaction effects in seedling deaths and number of days for infection under greenhouse conditions. Among the hosts, seedlings of coffee accessions P27 (Bonga), P34, P38 and P41 (Berhane-Kontir); and P47 and P49 (Yayu) showed significantly ($P < 0.05$) higher disease severity of 79.2, 76.5, 85.7, 72.8, 70.1 and 70.4 mean percent deaths, respectively (Table 3), with corresponding incubation periods ranging from 54.8 to 72.1 mean number of days (Table 4). Thus, they are broadly grouped as highly susceptible to the pathogen isolates. The seedlings of Harenna coffee accessions P4 and P6 expressed highly resistant reaction with significantly ($P < 0.05$) lower disease severity of about 22%. Harenna (P11) and Bonga (P17) seedlings appeared to be

Table 4. Incubation periods (mean number of days) for wilt symptom appearance on seedlings of forest coffee accessions inoculated with four *Gibberella xylarioides* isolates and grown under the greenhouse conditions at Jimma in 2010.

Forest coffee accession[1]	*Gibberella xylarioides* isolate[2]					
	B23	**SH21**	**Y21**	**H11**	**G11**	**Mean**
P4	28.3^{h-j}	0.0 ^{j3}	0.0^{j}	106.0^{a}	62.3^{c-h}	39.3^{D}
P6	56.3^{d-i}	0.0^{j}	23.7^{ij}	97.7^{a-c}	89.3^{a-}e	53.4^{CD}
P11	61.7^{c-i}	0.0^{j}	94.0^{a-d}	96.7^{a-c}	80.0^{a-g}	66.5^{A-C}
P17	61.0^{c-i}	28.3^{h-j}	80.0^{a-g}	80.7^{a-g}	69.0^{a-g}	63.8^{A-C}
P21	42.7^{g-i}	64.0^{b-h}	66.3^{b-h}	94.3^{a-d}	47.3^{f-i}	62.9^{A-C}
P27	57.0^{d-i}	71.0^{a-g}	73.3^{a-g}	66.7^{b-g}	61.7^{c-i}	65.9^{A-C}
P34	61.7^{c-i}	73.3^{a-g}	66.3^{b-h}	89.7^{a-}e	64.0^{b-h}	71.0^{AB}
P38	71.0^{a-g}	71.0^{a-g}	81.3^{a-f}	73.3^{a-g}	64.0^{b-h}	72.1^{A}
P41	66.7^{f-i}	49.7^{f-i}	61.7^{c-i}	83.0^{a-f}	57.0^{d-i}	63.6^{A-C}
P47	52.3e^{-i}	0.0^{j}	66.3^{b-h}	96.7^{a-c}	61.7^{c-i}	55.4^{A-D}
P49	57.0^{d-i}	0.0^{j}	71.0^{a-g}	75.0^{a-g}	71.0^{a-g}	54.8^{B-D}
P59	57.0^{d-i}	0.0^{j}	71.0^{a-g}	92.3^{a-d}	66.3^{b-h}	57.3^{A-C}
7440	80.0^{a-g}	0.0^{j}	88.7^{a-}e	101.3^{ab}	75.7^{a-g}	69.1^{A-C}
Means	57.9^{Y}	27.5^{X}	64.9^{Z}	88.7^{W}	66.9^{Z}	

[1]Coffee accessions P4, P6 and P11 selected from Harenna; P17, P21 and P27 from Bonga; P34, P38 and P41 from Berhane-Kontir; and P47, P49 and P59 from Yayu forests; and 7440 moderately resistant variety; [2]*Gibberella xylarioides* isolates coded as B23, SH21, Y21, H11 and G11 selected from Bonga, Berhane-Kontir, Yayu and Harenna forest coffee sites and Gera, respectively; [3]0.0 (zero value) indicates there was no seedling death during the study period (six months after inoculation). Means followed with the same letter(s) are not significantly (P < 0.05) different from each other according to DMRT. LSD values for the cultivars, the isolates and the interactions comparisons were 17.1, 10.6 and 38.3, respectively. CV = 28.75%.

moderately resistant as the standard check (*cv*. 7440) with respective severity levels of 33.6 and 35.7% (Table 3). Among the pathogen isolates, the most aggressive strain was 'H11' from Harenna forest coffee site in the southeast by causing significantly (P < 0.05) severe seedling death of 78.7%; followed by Bonga 'B23' and Yayu 'Y21' isolates collected in southwest forest coffee sites of Ethiopia. They were as aggressive as the strain isolated from plantation coffee at Gera 'G11' while Berhane-Kontir isolate 'SH21' was the least aggressive pathogen with 20.8% seedling death (Table 3).

The accession versus isolate interactions demonstrated differential effects, and thus, seedlings of Harenna accessions (P4, P6 and P11) were horizontally resistant (< 30%) to almost all isolates of *G*. *xylarioides* but susceptible to the isolate originated from the same site (H11) which induced 90% seedling death. In contrast, Yayu accessions (P47, P49 and P59) were highly resistant only to Berhane-Kontir isolate (SH21) without expressing any wilt symptoms (0.0%); although, they are susceptible to the other four isolates, namely, 'B23', 'Y21', 'H11' and 'G11' with varying disease levels (Table 3). With respect to the differential effect of the pathogen isolates, the Harenna isolate (H11) induced higher death rates (up to 90%) on all accessions including the moderately resistant check '*cv*. 7440' being horizontally pathogenic strain. On the other hand, the Berhane-Kontir isolate 'SH21' was not or weakly pathogenic to the seedlings of Harenna and Yayu accessions (P4 to P11, P47 to P59) and the check cultivar (*cv*. 7440) without any apparent symptoms of wilt (0.0%). The southwest forest coffee isolates 'B23' and 'Y21' showed similar aggressiveness as that of Gera strain 'G11' pathogenic to the seedlings of all coffee accessions. It was found in this study that, on average, isolate 'H11' with faster growth were more aggressive than those with slow growth rate agreeing with earlier report by Girma and Mengistu (2000).

The results of the present study are in line with the already documented findings on the coffee wilt pathosystem (Girma, 2004; Adugna et al., 2005; Girma et al., 2009b) that support the occurrence of predominantly horizontal resistance with few vertical type of reactions in the wild *Arabica* coffee populations. There exists correspondingly large variation in aggressiveness with little indication of virulence in the *G. xylarioides* strains in the forest coffee systems of Ethiopia. Earlier reports (Adugna et al., 2005; Girma et al., 2009b; Rutherford et al., 2009) evidenced that there is host specialization within *G. xylarioides* populations that might have attained pathogenic fitness through coevolution. In conclusion, *G. xylarioides* populations in the forest coffee systems are as pathogenic as those strains of the fungus in semi-forest, plantation and garden coffee production systems with certain variations in aggressiveness or virulence and cultural characteristics such as pigmentation and growth

nature. The fungus populations sampled in the forest coffee sites showed basically similar cultural and morphological characteristics of the species *G. xylarioides* Heim and Saccas (*F. xylarioides* Steyaert). However, the differences between southeast (Harenna) and southwest forest coffee sites (Bonga, Berhane-Konitr and Yayu) in colony growth, pigmentation and aggressiveness/pathogenicity indicate intraspecific subgroup in relation to co-adaptation to the various coffee types (*Arabica* coffee diversity) and ecological conditions in the region, that is, between the east and west of the Great Rift Valley.

The pathogen populations in most forest coffee areas are more aggressive or comparable to the strains in plantation, garden and semi-forest coffee production systems. In addition to susceptibility of the host and the dynamics of interacting factors including human activities or interference in the natural/wild ecosystems, the present increase of coffee wilt in the forest coffee sites can be attributable to aggressiveness/virulence of *G. xylarioides* strains. Thus, it is necessary to practice coffee wilt management options.

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