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Rep-PCR Genomic Fingerprinting Revealed Genetic Diversity and Population Structure among Ethiopian Isolates of *Pseudocercospora griseola* Pathogen of the Common Bean (*Phaseolus vulgaris* L.)

Yayis Rezene[1,2*], Kassahun Tesfaye[2,3], Mukankusi Clare[4], Allan Male[4] and Paul Gepts[5]

[1]*Molecular Biotech Laboratory, Southern Agricultural Research Institute, Awassa, Ethiopia*
[2]*Department of Microbial, Cellular and Molecular Biology, Addis Ababa University, Ethiopia*
[3]*Ethiopian Biotechnology Institute, Addis Ababa, Ethiopia*
[4]*Centro Internacional de Agricultura Tropical (CIAT), Kampala, Uganda*
[5]*Department of Plant Sciences, University of California, Shields Avenue, Davis, California, USA*

Abstract

Angular leaf spot (*Pseudocercospora griseola*) is one of the most devastating diseases affecting common bean production in most parts of Ethiopia. Thus, use of common bean varieties with durable resistance is the most effective and economical control measure. Knowledge about the genetic variability and population structure of the pathogen populations is important for a successful common bean improvement program. The objective of this study was to determine the genomic diversity existing among and between *P. griseola* isolates which were obtained from the field survey collection of diverse common-bean growing areas of Ethiopia. The study used the repetitive extragenic palindromic elements-polymerase chain reaction protocol to fingerprinting DNA sequence diversity. To study the genetic diversity, we analysed molecular data from 79 single-spore isolates of the *P. griseola* pathogen. Hence, Molecular Analysis of Variance (AMOVA) and cluster analysis revealed the existence of high genetic diversity within and among *P. griseola* isolates. ERIC PCR produced 21 different patterns of clusters, whereas, REP-PCR and BOX PCR produced 11 and 5 different patterns of clusters respectively. This is because of some isolates that shared the same BOX patterns could be distinguished by the ERIC and REP finger printing patterns. The ERIC-, BOX- and REP-PCR combined fingerprinting patterns discriminated 25 different patterns among the 79 monosporic *P. griseola* isolates were produced at cut-off 77% genetic similarity matrix. These discriminated clusters revealed the existence of genetic diversity within and among the isolates of *P. griseola* collected from the diverse common bean growing regions of Ethiopia.

Keywords: Repetitive extragenic palindromic elements; Polymerase chain reaction; Genetic characterization; Pathogen differentiation; *Pseudocercospora griseola*

Introduction

Common bean (*Phaseolus vulgaris* L.) is the most cultivated pulse crop worldwide. It is one of the major food and cash crops with a significant contribution to national economy and also traditionally ensures food security in Ethiopia [1]. Several biotic and abiotic stress are limiting the productivity of common bean of which Angular leaf spot (ALS) caused by *Pseudocercospora griseola* is the most devastating disease its yield reduction is estimated to reach 80% [2]. The use of resistance common bean varieties with durable resistance is the most effective and economical control measure of ALS. However, getting common bean varieties with durable resistance is not easy. Knowledge about the genetic variability of the pathogen populations is important for a successful common bean improvement program that aims to develop disease resistant varieties [3]. Genetic structure is defined as the amount and distribution of genetic variation within and among populations [4]. Thus, knowledge of genetic structure gives insight into the evolutionary processes that shaped a population in the past. It is useful to differentiate between the two types of genetic diversity that contribute to genetic structure: gene diversity and genotype diversity, Gene diversity increases as the number of alleles increases and the relative frequencies of those alleles become more equal. Genotype diversity refers to the number and frequencies of multi-locus genotypes, or genetically distinct individuals, in a population. Genotype diversity is an important concept for plant pathogens that have a significant component of asexual reproduction in their life history [3]. The genomes of microbes contain a variety of repetitive DNA sequences, accounting for up to 5% of the genome [5]. Many of these repetitive DNA elements are of unknown function and have been localized to both intergenic and extragenic regions of the microbial genome. The Palindromic Units (PU) or Repetitive Extragenic Palindromes (REP) constitutes the characterized family of bacterial repetitive sequences. PUs are present in about 500-1000 copies in the chromosome of *Escherichia coli* and of *Salmonella typhimurium*. PU sequences consist of a 35-40 bp inverted repeat and are found in clusters. A second family of repetitive elements, called IRU (Intergenic Repeat Units) or ERIC (Enterobacterial Repetitive Intergenic Consensus), has been described [6]. IRU are 124-127 bp long in which successive copies (up to six) are arranged in alternate orientation [7,8]. Both PU and IRU families are similarly located in non-coding, probably transcribed, regions of the chromosome. Repetitive Element Polymorphism PCR (rep-PCR) fingerprinting has become a frequent method to discriminate bacteria species analysing the distribution of repetitive DNA sequences in prokaryotic genomes [6]. Rep-PCR is based on the observation that outwardly facing oligonucleotide primers, complementary to interspersed repeated sequences, enable the amplification of differently sized DNA fragments, consisting of sequences lying between these elements [9]. Multiple amplicons of different sizes can be resolved by

***Corresponding author:** Yayis Rezene, Molecular Biotech Laboratory, Southern Agricultural Research Institute, Awassa, Ethiopia, Tel: +251-462206575; E-mail : rezene77@gmail.com

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electrophoresis, establishing DNA-fingerprint-specific patterns for bacterial strains [10]. Several of these interspersed repetitive elements are conserved in diverse genera of bacteria and, therefore, enable single primer sets to be used for DNA fingerprinting in many different microorganisms [9,10]. Although rep-PCR primers were developed for repetitive elements in prokaryotic genomes, these primers have been applied with success in the fingerprinting of eukaryotic genomes as well [11]. Thus, rep-PCR primers have been used to characterize the variability of several fungal genera [12-16]. Endogenous repetitive DNA elements have been identified in fungi and used to generate genomic fingerprints [11]. For example, repetitive sequences like microsatellites were shown to be useful targets for DNA-based typing because of their length variation and widespread occurrence [17]. Rep-PCR fingerprinting is a highly reproducible and simple method to distinguish closely related microbial strains, deduce phylogenic relationships, and study their diversity in different ecosystems [18]. However, few studies have been performed regarding the applicability of rep-PCR to the discrimination of fungal species. Currently, rep-PCR has been proved as a useful molecular method to identify and study the genetic variability in the fungal species. The aim of the work presented here was to study the genetic diversity and the population structure of *P. griseola* isolates obtained from the collected infected leaves of common bean from the various areas of Ethiopia using rep-PCR molecular finger printing methods. To the best of our knowledge, this is the first report of the use of rep-PCR genetic fingerprinting to study the genetic diversity of *P. griseola* from Ethiopia.

Materials and Methods

Sample collection and isolation of *Pseudocercospora griseola*

The experiment was conducted in the Molecular Biotech Lab at the Southern Agricultural Research Institute (SARI), Hawassa, Ethiopia. Leaves with lesions of ALS were sampled and collected from fields of common bean during the field survey in 2016 and 2017 from diverse agroecological zones of Ethiopia that are known major common bean production areas (Figure 1). A total of 78 pure and single spores were isolated from infected and diseased leaves collected from the various common-bean growing regions using methods developed by CIAT (Table 1). Moreover, one additional characterised isolate from Andean gene pools that was obtained from CIAT-Uganda was included in the study to differentiate the Ethiopian isolates into Middle American and Andean groups. Isolation and monosporic culture were done according to the method developed by Pastor-Corrales et al. [19]. Accordingly, freshly infected leaves of common bean were used and single spore were transferred from fungal structures formed on lesions to culture media, using a sterilized fine needle under a dissecting microscope (Motic compound microscope). Monosporic cultures of *P. griseola* were grown on V8 culture media in 12 h dark and light incubator for 20 days at 25°C until genomic DNA extraction.

Genomic deoxyribonucleic acid (DNA) extraction

Genomic DNA was extracted using a protocol described by Mahuku et al. [20-22] with minor modification. The harvested fresh fungal mycelium was transferred to sterilized 1.7 ml tube containing 500 microliter of TES extraction buffer (0.2 m Tris-HCl pH 8.0; 10 mm EDTA, pH 8.0, 0.5 m NaCl, 1% SDS); sterilized sand was added and grinded using mortar and pistil. The samples were mixed using vortex for 30s and then incubated in the water bath at 65°C for 30 minutes before it was centrifuged for 15 minutes at 20,800 g. The supernatant was transferred to a new tube and an equal volume of ice cold isopropanol was added. Tubes were then incubated at -20°C for 1 hour, followed by centrifugation for 10 minutes at 20,800 g to pellet the DNA. The supernatant was eliminated and the DNA pellet was washed with 800 microliters of cold 70% ethanol; the tubes were then turned upside down on clear sterile paper towel for 45 minutes to air dry. The dried DNA pellet was diluted with 1 x TAE buffer.

Rep-PCR fingerprinting

In rep-PCR, fingerprinting three families of repetitive sequences were used (Table 2). They included:

1) The Repetitive Extragenic Palindromic (REP) sequence REP1R-1/ REP2-1 (18 nucleotides in length), as described by Versalovic et al. [6].

2) The Enterobacterial Repetitive Intergenic Consensus (ERIC) in which two oligonucleotide primer pairs used for PCR amplification ERIC1R/ERIC2 (22 nucleotide in length).

3) BOX elements (22 nucleotide in length) [23].

Optimal PCR conditions for each of the primer sets used were as described by De-Bruijn [9] with minor modification of the annealing temperature. The reproducibility of rep-PCR was tested by amplifying DNA two times from ten randomly chosen strains. The PCR amplifications were performed with a thermal cycler 2710 using PCR premix (GEillustra) as described by the manufacturer [24]. The PCR products were electrophoresed in a 1.5% agarose gel for 2 h at a constant voltage of 90 V in 1 × TAE buffer (40 mMTris-Acetate, 1 mM EDTA, pH 8.0) at 4°C. Gels were stained in ethidium bromide and the rep-PCR profiles and fingerprinting patterns were visualized under UV light, and the image was captured using a Canon digital camera mounted on the visualization hood.

Data analysis and interpretation

Analysis of genetic similarity and dissimilarity: The results of PCR fingerprinting with ERIC, BOX, and REP primers were collected into matrices with scored presence (1) or absence (0), of banding pattern in each PCR analysis lane. In each case, a simple matrix was obtained by comparing pairs of isolates of *P. griseola* using a simple matching coefficient (SM). A dendrogram was constructed with all parameters together after cluster analysis with the Dice similarity matrix, the Jaccard dissimilarity matrix and the Euclidean distance [25]. As suggested by the Kosman diversity and distance measures [26] for populations with an asexual and mixed mode of reproduction were considered in this specific study to measure the genetic diversity with populations and distance between populations. The Kosman distance and diversity measures for populations were calculated using different measures of dissimilarity between individuals (the simple mismatch, Jaccard, and Dice coefficients of dissimilarity). Similarity among the profiles was calculated using the Dice similarity matrix. The clustering was based on an average linkage or unweighted pair group method with arithmetic averages (UPGMA).

Analysis of molecular variance (AMOVA) and genetic diversity: An analysis of molecular variance (AMOVA) was performed using GenAlEx6.1 [27] to assess genotypic variations across all the populations studied. The analysis included partitioning of total genetic variation into within-groups and among groups variance components, hence, it provided a measure of intergroup genetic distance as proportion of the total variation residing among populations. The significance of analysis was tested using 999 random permutations.

Results

Analysis of molecular markers

Rep-PCR amplification in *Pseudocercospora griseola*: Rep-PCR analysis using primer sets REP, ERIC and BOX of highly conserved repetitive sequences resulted in differential banding patterns among and within *P. griseola* populations collected from the diverse common bean growing regions of Ethiopia. In Rep-PCR, three families of repetitive sequences were used, including the repetitive extragenic palindromic (REP) sequences, enterobacterial repetitive extragenic palindromic sequence, enterobacterial repetitive intergenic consensus (ERIC), and BOX elements [23]. Amplification of genomic DNA from the *P. griseola* isolates collected from the diverse common bean growing

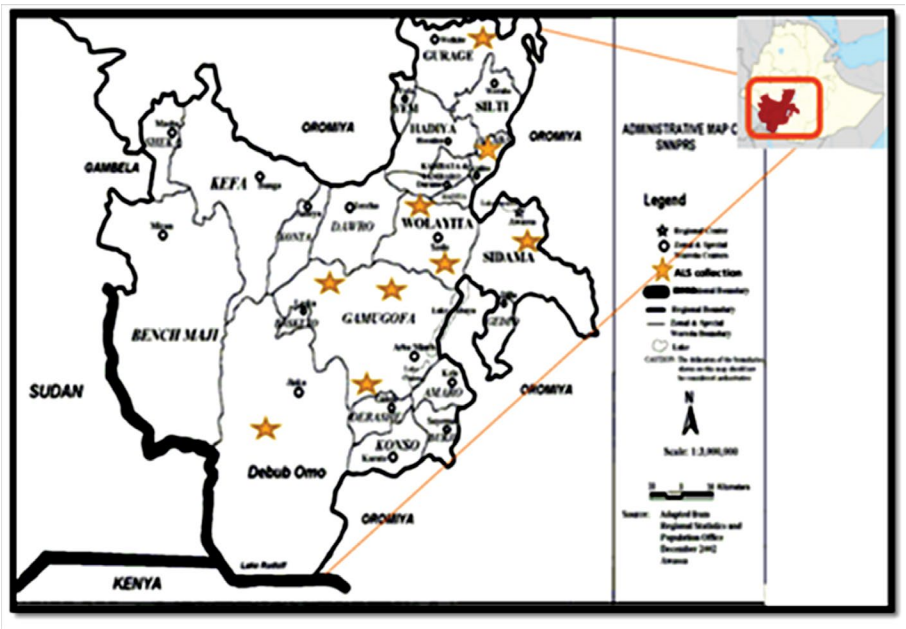

Figure 1: Geographic location of Pseudocercospora griseola sampling sites in the study on the map of Ethiopia.

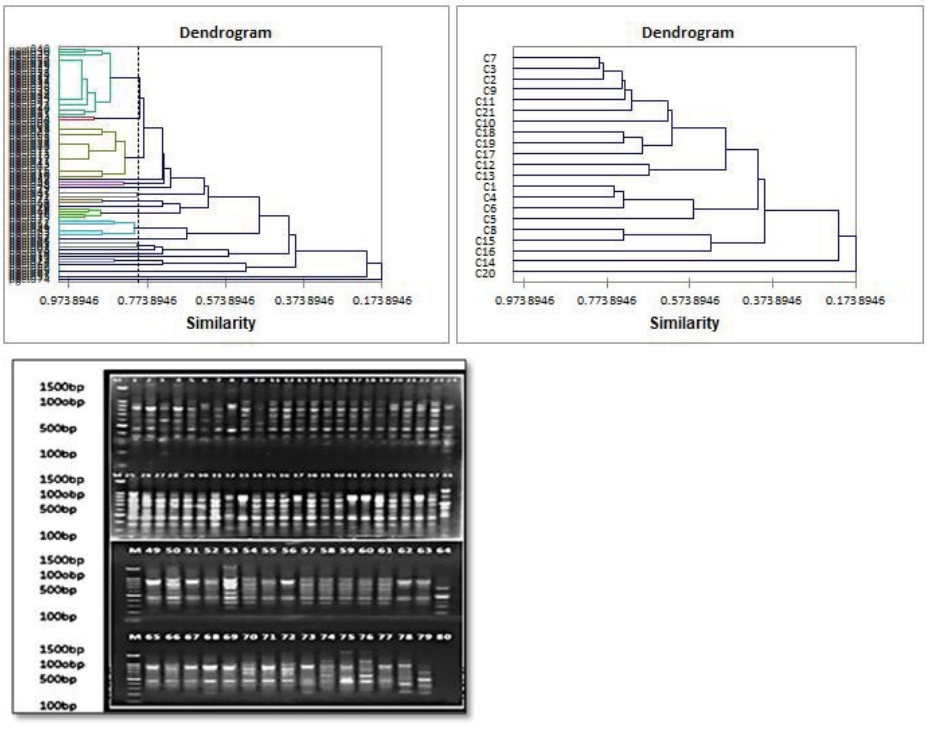

Figure 2: Agarose gel showing polymerase chain reaction genomic fingerprinting pattern and cluster analysis based on UPGMA and Dice similarity coefficients with ERIC-PCR gDNA extracted by the Mahuku (2004) method from 79 (1-79) single spore isolates of *Pseudocercospora griseola*. The isolates were collected from the diverse common bean growing agro-ecologies of Ethiopia. M=100 bp genetic marker.

regions of Ethiopia with rep-PCR resulted in complex fingerprint patterns (Figures 2-4). Rep-PCR fingerprint patterns for isolates of *P. griseola* were examined. The size of amplification products ranged from 100bp to 1500bp. Analysis of the ERIC PCR fingerprinting patterns by UPGMA using Dice similarity coefficient resulted 21 distinct groups among the 79 *P. griseola* at 77% similarity cut of level (Figure 2). While BOX and REP PCR fingerprinting pattern discriminated 5 and 11 distinct groups among the 79 *P. griseola* at cut-off 60 and 66% similarities level respectively (Figures 3 and 4). Hence, ERIC-PCR was the most informative to differentiate isolates of *P. griseola* collected from the diverse common bean growing regions of Ethiopia. The dendrogram obtained from the cluster analysis using combined ERIC-, REP- and BOX-PCR genomic fingerprints revealed the overall grouping of the *P. griseola* isolates collected from the diverse areas of Ethiopia. Thus, combined REP-PCR fingerprinting discriminated 25 distinct groups among the 79 isolates of *P. griseola* at a cut off 77% similarity molecular level (Figure 5). Previously the distribution of ERIC, REP and BOX elements has been examined and reported in diverse prokaryotic genomes [6]. Previous report of repetitive PCR primers matching with these repetitive sequences has been described for the molecular characterization of bacterial strains [9,10]. Our results were consistent with many reports that indicates these repetitive elements, which are highly conserved in the bacterial kingdom, are also presents in the fungus *P. griseola* populations collected from diverse bean growing regions of Ethiopia, which allowed us to differentiate the 79 isolates of *P. griseola*.

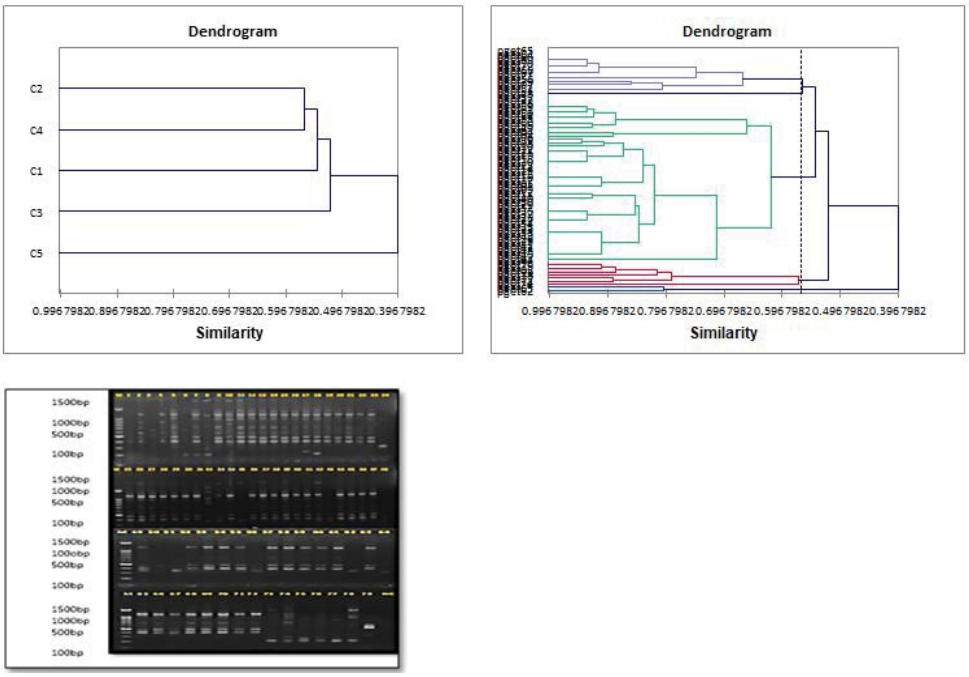

Figure 3: Agarose gel showing polymerase chain reaction (PCR) and genomic fingerprinting pattern and cluster analysis based on UPGMA and Dice similarity coefficients with BOX -PCR from the gDNA extracted by the Mahuku (2004) method from 79 (lane 1 to 79) single spore isolates of Pseudocercospora griseola collected from diverse common bean growing regions of Ethiopia. M: genetic marker 100bp, NC: Negative Control.

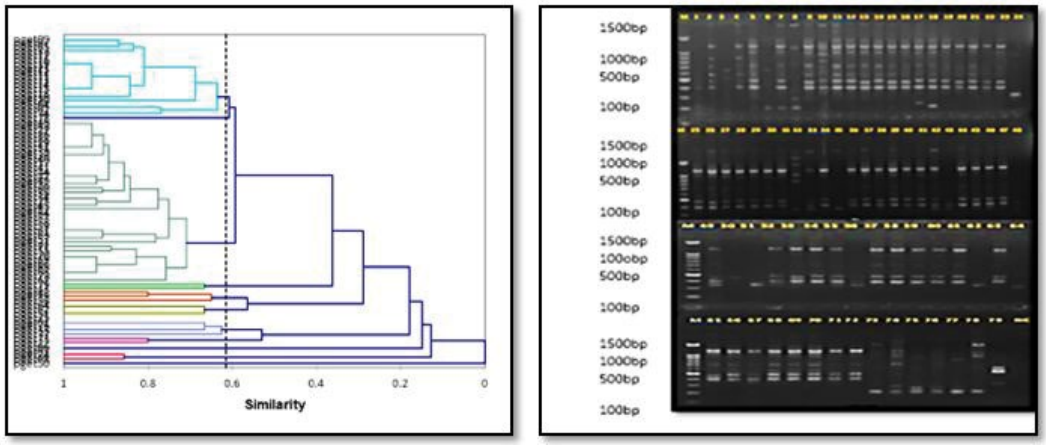

Figure 4: Agarose gel showing polymerase chain reaction produced genomic fingerprinting pattern and Cluster analysis based on UPGMA and Dice similarity coefficients with REP-PCR from 79 (lane 1-79) single spore isolates of *Pseudocercospora griseola* collected from diverse common bean growing regions of Ethiopia. M: molecular marker 100bp, NC; Negative Control.

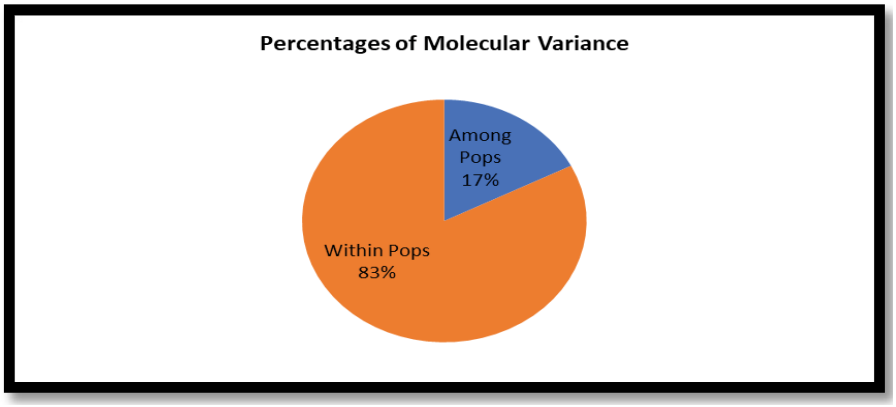

Figure 5: Pie chart indicating the percentage of molecular variance partition.

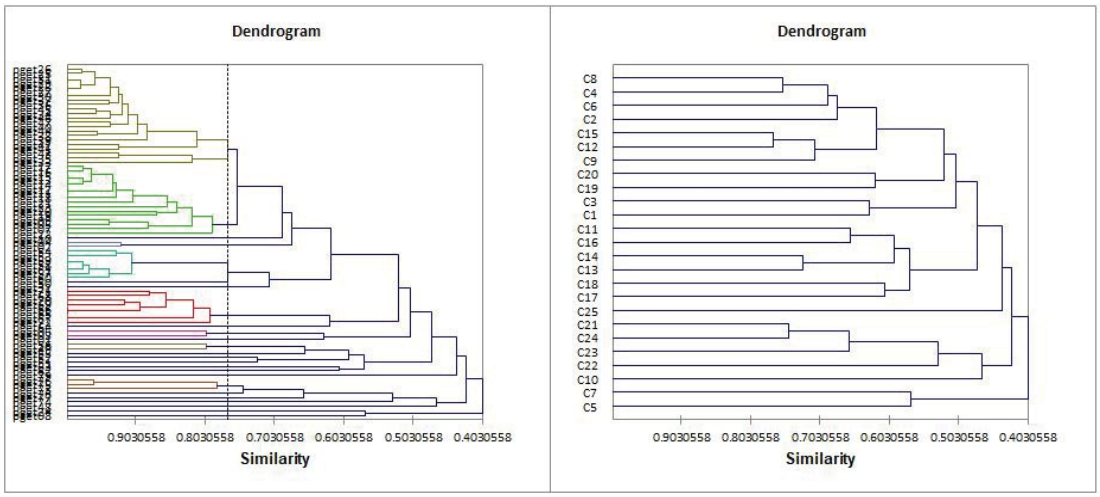

Figure 6: Cluster analysis based on UPGMA and Dice similarity coefficients obtained from the combined REP, BOX and ERIC genomic fingerprinting patterns of 79 single-spore isolates of Pseudocercospora griseola collected from diverse common bean growing regions of Ethiopia. Same colors within the cluster indicates genetic similarity of *P. griseola* isolates and the dendrogram in the right side indicates the genetically discriminated 25 clusters among the 79 monosporic isolates.

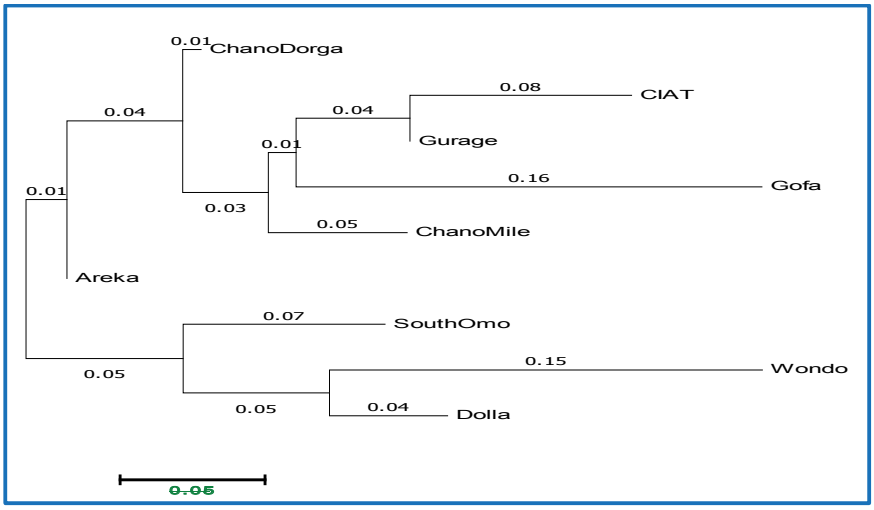

Figure 7: The evolutionary history was inferred using the Minimum Evolution method. The optimal tree with the sum of branch length=0.75085937 is shown. The tree is drawn to scale, with branch lengths (next to the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The ME tree was searched using the Close-Neighbour-Interchange (CNI) algorithm at a search level of 1. The Neighbour-joining algorithm was used to generate the initial tree. Evolutionary analyses were conducted in MEGA6.

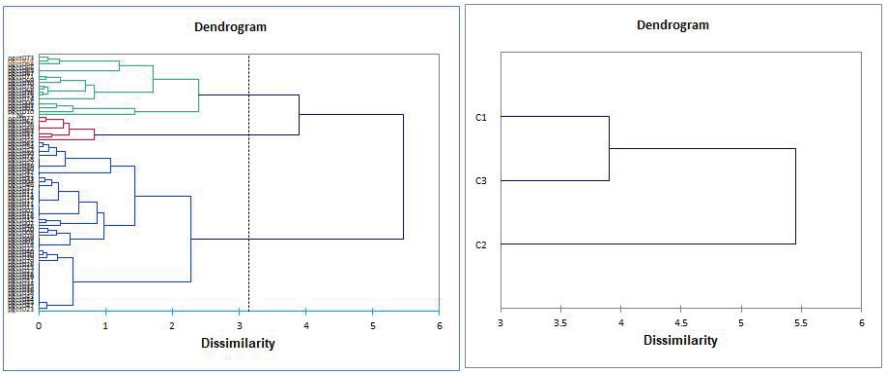

Figure 8: Cluster analysis dendrogram of 79 **Pseudocercospora griseola** isolates collected from the diverse common bean growing areas of Ethiopia using rep-PCR molecular fingerprinting data.

CODE	Location	Altitude	Host	Genepool	Origin	Year of Collection
pget001	WONDO	1742 masl	PIC6	A	Ethiopia	2017
pget002	WONDO	1742 masl	ADP-0100	A	Ethiopia	2017
pget003	GOFA	1400 masl	SMALL RED	M	Ethiopia	2017
pget004	WONDO	1742 masl	ADP-0095	A	Ethiopia	2017
pget005	WONDO	1742 masl	ADP-0468	A	Ethiopia	2017
pget006	GOFA	1400 masl	SMALL RED	M	Ethiopia	2017
pget007	HALABA	1872 masl	TATU	A	Ethiopia	2017
pget008	GOFA	1400 masl	HDUME	M	Ethiopia	2017
pget009	WONDO	1742 masl	ADP-0668	A	Ethiopia	2017
pget010	WONDO	1742 masl	ADP-0518	A	Ethiopia	2017
pget011	WONDO	1742 masl	ADP-0037	A	Ethiopia	2017
pget012	WONDO	1742 masl	ADP-0037	A	Ethiopia	2017
pget013	WONDO	1742 masl	ADP-0675	A	Ethiopia	2017
pget014	WONDO	1742 masl	ADP-0675	A	Ethiopia	2017
pget015	WONDO	1742 masl	ADP-0675	A	Ethiopia	2017
pget016	WONDO	1742 masl	ADP-0675	A	Ethiopia	2017
pget017	DOLLA	1865 masl	RED WOLAITA	M	Ethiopia	2017
pget018	DOLLA	1865 masl	RED WOLAITA	M	Ethiopia	2017
pget019	DOLLA	1865 masl	RED WOLAITA	M	Ethiopia	2017
pget020	DOLLA	1865 masl	RED WOLAITA	M	Ethiopia	2017
pget021	DOLLA	1865 masl	RED WOLAITA	M	Ethiopia	2017
pget022	DOLLA	1865 masl	RED WOLAITA	M	Ethiopia	2017
pget023	DOLLA	1865 masl	RED WOLAITA	M	Ethiopia	2017
pget024	DOLLA	1865 masl	RED WOLAITA	M	Ethiopia	2017
pget025	DOLLA	1865 masl	RED WOLAITA	M	Ethiopia	2017
pget026	DOLLA	1865 masl	RED WOLAITA	M	Ethiopia	2017
pget027	DOLLA	1865 masl	RED WOLAITA	M	Ethiopia	2017
pget028	DOLLA	1865 masl	RED WOLAITA	M	Ethiopia	2017
pget029	DOLLA	1219 masl	NASIER	M	Ethiopia	2017
pget030	CHANO DORGA	1219 masl	NASIER	M	Ethiopia	2017
pget031	SOUTH OMO	1363 masl	RED WOLAITA	M	Ethiopia	2017
pget032	SOUTH OMO	1363 masl	RED WOLAITA	M	Ethiopia	2017
pget033	SOUTH OMO	1363 masl	H DUME	M	Ethiopia	2017
pget034	SOUTH OMO	1363 masl	H DUME	M	Ethiopia	2017
pget035	SOUTH OMO	1363 masl	H DUME	M	Ethiopia	2017
pget036	SOUTH OMO	1363 masl	H DUME	M	Ethiopia	2017
pget037	SOUTH OMO	1363 masl	H DUME	M	Ethiopia	2017
pget038	CHANO MILE	1219 masl	NASIER	M	Ethiopia	2017
pget039	BAKO GAZAR	1363 masl	SMALL RED	M	Ethiopia	2017
pget040	BAKO GAZAR	1363 masl	SMALL RED	M	Ethiopia	2017
pget041	SOUTH OMO	1363 masl	HDUME	M	Ethiopia	2017
pget042	AREKA	1802 masl	ADP	A	Ethiopia	2017
pget043	AREKA	1802 masl	ADP	A	Ethiopia	2017
pget044	CHANO DORGA	1219 masl	NASIER	M	Ethiopia	2017

pget045	CHANO DORGA	1219 masl	NASIER	M	Ethiopia	2017
pget046	CHANO DORGA	1219 masl	NASIER	M	Ethiopia	2017
pget047	CHANO DORGA	1219 masl	NASIER	M	Ethiopia	2017
pget048	CHANO DORGA	1219 masl	NASIER	M	Ethiopia	2017
pget049	CHANO DORGA	1219 masl	NASIER	M	Ethiopia	2017
pget050	CHANO DORGA	1219 masl	NASIER	M	Ethiopia	2017
pget051	CHANO DORGA	1219 masl	NASIER	M	Ethiopia	2017
pget052	CHANO DORGA	1219 masl	NASIER	M	Ethiopia	2017
pget053	CHANO DORGA	1219 masl	NASIER	M	Ethiopia	2017
pget054	CHANO DORGA	1219 masl	NASIER	M	Ethiopia	2017
pget055	CHANO DORGA	1219 masl	NASIER	M	Ethiopia	2017
pget056	CHANO DORGA	1219 masl	NASIER	M	Ethiopia	2017
pget057	CHANO DORGA	1219 masl	NASIER	M	Ethiopia	2017
pget058	CHANO DORGA	1219 masl	NASIER	M	Ethiopia	2017
pget059	CHANO MILE	1212 masl	NASIER	M	Ethiopia	2017
pget060	CHANO MILE	1219 masl	NASIER	M	Ethiopia	2017
pget061	CHANO MILE	1219 masl	NASIER	M	Ethiopia	2017
pget062	CHANO MILE	1219 masl	NASIER	M	Ethiopia	2017
pget063	CHANO MILE	1219 masl	NASIER	M	Ethiopia	2017
pget064	CHANO MILE	1219 masl	NASIER	M	Ethiopia	2017
pget065	CHANO MILE	1219 masl	NASIER	M	Ethiopia	2017
pget066	CHANO MILE	1212 masl	NASIER	M	Ethiopia	2017
pget067	CHANO MILE	1212 masl	NASIER	M	Ethiopia	2017
pget068	CHANO MILE	1212 masl	NASIER	M	Ethiopia	2017
pget069	CHANO MILE	1212 masl	NASIER	M	Ethiopia	2017
pget070	HALABA	1872 masl	HDUME	M	Ethiopia	2016
pget071	GURAGE	1604 masl	NASIER	M	Ethiopia	2016
pget072	GURAGE	1770 masl	NASIER	M	Ethiopia	2016
pget073	GURAGE	1772 masl	NASIER	M	Ethiopia	2016
pget074	AREKA	1884 masl	RED WOLAITA	M	Ethiopia	2016
pget075	GURAGE	1742 masl	NASIER	M	Ethiopia	2016
pget076	KAO60 /CIAT	--	--	A	--	2016
pget077	240	--	--	--	--	2016
pget078	220	--	--	--	--	2016
pget079	224	--	--	--	--	2016

Table 1: *Pseudocercospora griseola* isolates collected from diverse common bean growing regions of Ethiopia.

Genetic markers	Sequences 5' to 3'	T_a °C	GC%	Number of nucleotides	References
REP 1R	IIIICGICGICATCIGGC	49	52.9	18	[21]
REP 2	IIICGNCGNCATCNGGC	58	52.9	17	[21]
ERIC 1R	ATGTAAGCTCCTGGGGATTCAC	58	50	22	[22]
ERIC 2	AAGTAAGTGACTGGGGTGAGCG	42	54.5	22	[22]
BOX A1R	CTACGGCAAGGCGACGCTGACG	50	68.2	22	[6]

Table 2: Molecular markers used to amplify PCR fingerprinting products of *Pseudocercospora griseola*.

Analysis of molecular variance (AMOVA): The analysis of molecular variance (AMOVA), which revealed 83% and 17% genetic variations ($p<0.05$) within and among the monosporic isolates of *P. griseola* obtained from the collections of the diverse common bean growing areas of Ethiopia (Figures 5-8).

Cluster analysis of BOX, REP and ERIC-PCR fingerprinting pattern: Cluster analysis was performed on the combined DNA fingerprints produced from BOX, REP and ERIC PCR products (Figure 5). The dendrogram obtained from the cluster analysis of combined (REP/BOX/ERIC) Rep-PCR fingerprinting patterns discriminated the entire monosporic *P. griseola* isolates, that were collected from various common bean regions of Ethiopia into 25 distinct types among the 79 *P. griseola* isolates. The results of the present study determined primarily the usefulness of Rep-PCR genomic fingerprinting as complimentary or as an alternative strategy to other methods of genomic diversity study of *P. griseola* isolates of the angular leaf spot of the common beans.

Discussion

The genomic DNA fingerprinting patterns found among the P.griseola isolates were found to be varied in size and number depending on each *P. griseola* isolates indicating the existence of diverse genetic variability within each isolate. However, some of the isolates showed similar DNA fingerprinting patterns with only minor differences; hence, these isolates with similar genomic DNA fingerprinting pattern were clustered in the same group. The Rep-PCR primers set families of ERIC, REP and BOX generated multiple distinct DNA genomic fingerprints ranging from 100 bp to 1500 bp (Figures 1-3). The results of genomic DNA fingerprint profiles obtained from monosporic isolates complement with the many of the previous reports and can be reproducible from one experiment to another [13,14,16,17]. The observed significant variation within the monosporic isolates of *P. griseola*, among the isolates of the same geographic locations were due to the co-exitance of diverse host genotypes and based on

many of the reports the pathogen might undergone parasexual that facilitates exchange of genetic material within and between isolates. It might also because of chromosomal inversion, deletion and presence of transposons because all are reported to have capability to increase the variability in *P. griseola* [28,29]. The genetic structure of *P. griseola* revealed no geographical differentiation. The small reds & white coloured beans from the Mesoamerican gene pool have been predominantly cultivated in Ethiopia with the exception of a few areas known for the cultivation of large and speckled red beans from the Andean gene pool [1].

Therefore, geographical specialization was not evident. This has important implications for the deployment of angular leaf spot resistance genes and the development of common bean cultivars for the ALS disease resistance. High genetic variability of *P. griseola* was observed in areas typically cultivating Mesoamerican common bean. Since Mesoamerican common beans are predominantly cultivated in Ethiopia, the greatest challenge to manage angular leaf spot of the common bean is in areas that are known for the cultivation of beans from the Mesoamerican gene pools. The lack of isolation by distance among the isolates of *P. griseola* from the diverse common bean growing areas of Ethiopia indicates the *P. griseola* fungi have efficient dispersal at the common bean growing areas of the region. From our study we confirmed that the genetic divergence between the populations was very low which was 13% whereas, 87% of the molecular variance was attributed to the variation within populations this was indicated with sharing of rep-PCR genomic finger printing pattern between geographic populations from distinct locations of common bean growing areas of Ethiopia, (for example genomic finger printing patterns between Dolla and south Omo the two locations are far away about 450 km from each other). The observed gene flow and sharing same genomic fingerprints between isolates of the two distinct locations could be due to different possibilities; one of the possibilities for the long-distance gene flow might be due to the long-distance gene flow nature of the pathogen and due to spore dispersal without human interference because of the wind and other natural influence. Moreover, the long distance geneflow over hundreds to thousands of kilometers has been reported in many of fungi [30] or the other possibility for the long-distance gene flow could be also due to the human involvement and the seed born nature of *P. griseola.* The informal seed system, which is common practice and is associated with movement of infected planting materials between different locations or common bean growing areas, including wind dispersal could be the main causes for the observed genomic fingerprinting pattern between distinct locations. This was explained with the presence of *P. griseola* isolates from different geographical regions in the same branch of the dendrogram. Human activities were reported and found to be responsible for the long-distance dispersal of may fungi and pathogens [31-33]. This study is the first report using rep-PCR genomic finger printing on genomic variation and population structure of *P. griseola* isolates that were collected from diverse common bean growing regions of Ethiopian. The results revealed that *P. griseola* in Ethiopia demonstrates with high level of genomic diversity. As previously reported, Rep-PCR fingerprinting was a highly reproducible and a simple method to distinguish closely related fungal isolates. To infer the phylogenic relationships and to study their diversity in different ecosystems [9,19]. The majority of our *P. griseola* samples were from the southern parts Ethiopia which is known for its wider and potential common bean production areas of Ethiopia. The area is known for its hotspot area for the angular leaf spot and majority of the isolates of *P. griseola* from this area were confirmed to be genetically very diverse and this area might not represent other parts of Ethiopia. The analysis of additional samples from other areas as well as more genes might allow defining the population structure of *P. griseola* existing in Ethiopia. We believe this study represents an important step towards understanding the presence of high genetic diversity within the *P. griseola* existing in common bean production areas of Ethiopia. Hence, the common bean breeding program aiming to develop durable resistance varsities should consider this information during the deployment of resistance genes to develop resistance common bean varieties.

Conclusion

This study was the first report on the genomic variation and population structure of *P. griseola* that were collected from the diverse common bean growing regions and the result revealed that *P. griseola* in Ethiopia displays with high level of genomic diversity. The genetic structure of *P. griseola* reveals no geographic differentiation. Moreover, the result from this specific study compliments with many of the reports that confirms the sources of genomic variability existed within and among the monosporic isolates of *P. griseola* obtained from the diverse common bean growing areas of Ethiopia might be the informal seed system that was dominantly practiced with common bean seed system within the small-scale farming community. Additionally, the movement of infected planting materials between different locations and wind dispersal of spores could be the main contributors to the presence of P. griseola isolates from different geographic regions in the same group. This could be also the main reason for the absence of geographic differentiation between common bean growing locations. As disease management strategy common bean seed multipliers and should give attention to produce pathogen free clean seed common bean for wider dissemination. Rep-PCR fingerprinting was a highly reproducible and a simple method to distinguish closely related fungal isolates. The regional and national common bean improvement programs in Ethiopia should also give priorities for gene deployment and marker aided gene pyramiding techniques in developing broad and multiple disease resistance common bean varieties along with identification of new sources of resistance common bean cultivar.

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