Genetic Characterization of *Cercospora sorghi* from Cultivated and Wild Sorghum and Its Relationship to Other *Cercospora* Fungi

P. Okori, P. R. Rubaihayo, A. Ekwamu, J. Fahleson, and C. Dixelius

First, fourth, and fifth authors: Department of Plant Biology, Swedish University of Agricultural Sciences, P.O. Box 7080, S-750 07 Uppsala, Sweden; and first, second, and third authors: Department of Crop Science, Makerere University, P.O. Box 7062, Kampala, Uganda.
Accepted for publication 19 February 2004.

ABSTRACT

Okori, P., Rubaihayo, P. R., Ekwamu, A., Fahleson, J., and Dixelius, C. 2004. Genetic characterization of *Cercospora sorghi* from cultivated and wild sorghum and its relationship to other *Cercospora* fungi. *Phytopathology* 94:743-750.

Genetic variability and population structure of *Cercospora sorghi* from wild and cultivated sorghum were investigated to gain insight into their potential impact on epidemics of gray leaf spot of sorghum in Africa. Population structure was examined using data derived from amplified fragment length polymorphism (AFLP) of *C. sorghi* by Nei's test for population differentiation, G_{ST} , and analysis of molecular variation (AMOVA). Two ecological populations of *C. sorghi* in Uganda were devoid of population structure ($G_{ST} = 0.03$, $\phi F_{ST} = 0.01$, $P = 0.291$). AMOVA revealed

that genetic variability was due mainly to variations within (99%) rather than between (0.35%) populations, and Nei's genetic distance between the two populations was 0.014. Phenetic analysis based on AFLP data and polymerase chain reaction–restriction fragment length polymorphism analyses of the internal transcribed spacer regions of rDNA and mitochondrial small subunit rDNA separated *Cercospora* cereal pathogens from dicot pathogens but did not differentiate among *C. sorghi* isolates from wild and cultivated sorghum. Our results indicate that Ugandan populations of *C. sorghi* compose one epidemiological unit and suggest that wild sorghum, while not affecting genetic variability of the pathogen population, provides an alternative host for generating additional inoculum.

Additional keyword: Sorghum bicolor.

Sorghum (*Sorghum bicolor* (L.) Moench) is an important staple cereal in many parts of Africa. Production is reduced by several diseases, including gray leaf spot caused by *Cercospora sorghi* Ellis & Everh., (8,9,23,29,32). Yield losses due to gray leaf spot of up to 67% have been recorded in some susceptible cultivars (23). Gray leaf spot of sorghum was reported in Africa in the 1950s (7,20) and, since then, epidemics have occurred sporadically and, in some cases, have been widespread (11,23,24,40). The factors accounting for the pattern of epidemics in this pathosystem are not known. In sub-Saharan Africa, sorghum generally is intercropped in the drier regions, and this may influence epidemics by reducing infection and sporulation efficiency (1, 15,17,34). With gray leaf spot of maize, recent studies have shown that variability in severity and incidence of the disease is attributed in part to pathogen variability and environmental factors (6). This variability also may apply to sorghum because, ultimately, environmental effects or cultivation practices influence selection pressure, which in turn affect pathogen genetic structure and disease epiphytotics (17,26). Genetic variability of *C. sorghi* populations is a possible contributor to the patterns of gray leaf spot epidemics, but there is paucity of such information (9). *C. sorghi* also infects wild sorghum lines such as *S. bicolor* var. *verticilliflorum* (Steud.), *S. bicolor* var. *arundinacium*, and *S. halapense* (7). *S. bicolor* var. *verticilliflorum* is the predominant wild sorghum subspecies in sub-Saharan Africa, and believed to be the progenitor of cultivated sorghum (8,19). The role of wild relatives in gray leaf spot epiphytotics is not known. In other pathosystems, wild host plants influence disease epidemics (5). Thus, a role of wild sorghum in gray leaf spot epidemics cannot be dismissed. Sorghum most probably was domesticated in Africa

(8,19); therefore, the *C. sorghi* pathosystem presumably has evolved over a long time in the region. Therefore, genetic analysis of *C. sorghi* collected from both wild and cultivated sorghum may help to characterize diversity and overall structure of the pathogen population and explain the role of these factors in epiphytotics in Africa.

Genetic analysis of pathogen populations for variability has been done mainly by investigating population structure. Population structure refers to the amount of and distribution of genetic variation within and among populations and is estimated using Wright's *F* statistic, designated as F_{ST} (45), G_{ST} (28), or ϕF_{ST} (12). Other measurements of genetic variability include estimates of genetic distance between populations (28), gene diversity or average heterozygosity (diversity at individual loci), and genotype diversity, based on the number of distinct individuals in a population (26). Gene diversity is more appropriate for asexually reproducing fungal species such as the *Cercospora* spp. These genetic tools have been used in several fungal species (26) and, thus, could be used to elucidate genetic variability of *C. sorghi*. The population studies of *C. sorghi* will elucidate the evolutionary responsiveness of the pathogen to previous selection pressures. Genetic study of *C. sorghi* is of interest due to this species phylogenetic relatedness to *C. zeae-maydis*, the cause of gray leaf spot in maize (7,18,42). Our results from population studies of East African *C. zeae-maydis* isolates indicated the absence of any population structure (30,31). Therefore, comparison of these results with *C. sorghi* genetic structure may elucidate the evolutionary history and potential of *C. sorghi* to develop into novel genotypes.

Genetic information required to design and deploy disease management tools can be obtained using a wide array of molecular techniques, including the amplified fragment length polymorphism (AFLP) analysis (26). AFLP previously was used to characterize *C. zeae-maydis* populations (10,31,42). Objectives of the present investigation were to (i) examine the population struc-

Corresponding author: P. Okori; E-mail address: Patrick.Okori@vbsg.slu.se

Publication no. P-2004-0517-01R

© 2004 The American Phytopathological Society

ture of Ugandan *C. sorghi* using AFLP, and (ii) investigate the genetic relatedness of *C. sorghi* to other *Cercospora* spp. Polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analyses of the internal transcribed spacer (ITS) regions of rDNA and mitochondrial small subunit (mtSSU) rDNA also were performed. Previous population analysis of East African *C. zea-maydis* populations showed the absence of a population structure between isolates collected from Uganda, Kenya, Rwanda, and Zimbabwe and no population structure was detected among isolates collected from the same country (30,31). These results are similar to those obtained in the present investigation, in which genetic structures of *C. sorghi* populations collected from Uganda were found, suggesting similar evolutionary history of the two pathogens. No genetic differences between *C. sorghi* haplotypes derived from wild or cultivated sorghum were observed.

MATERIALS AND METHODS

Sampling, fungal culture, and DNA isolation. Diseased leaves were collected from seven districts of Uganda, where sorghum is cultivated or the wild sorghum (*S. bicolor* var. *verticilliflorum* (Steud.) is prevalent (Fig. 1; Table 1). Samples were collected from sorghum fields and wild sorghum populations near fields in two main agroecological zones. The two agroecologies

were selected because of differences in climate and farming systems. Districts of Lira, Apac, and Masindi constitute one agroecological zone, the northern, moist farmlands. The region is located at an elevation of 1,024 m above sea level, with a mean temperature of 20°C and annual precipitation of 1,200 mm received during one long rain season (44). The districts of Mbale, Sironko, Mpigi, and Masaka constitute the second agroecological zone, comprising two production zones, the Jinja-Mbale farmlands and the Lake Victoria crescent. The Jinja-Mbale farmlands are located at an elevation of 1,213 m above sea level, while Lake Victoria crescent lies at an elevation of 1,174 m above sea level. Both areas have a mean temperature of 20°C and receive an annual precipitation of 1,200 mm during two annual rain seasons (44). Due to the patchy distribution of wild sorghum populations, two to three leaf samples were obtained per sampling site. Approximately five samples were collected from different sorghum fields. The leaf samples were air dried for 4 to 7 days and then stored at room temperature. A lesion was cut out from each leaf sample, surface sterilized using 0.5% sodium hypochlorite solution, rinsed twice in sterile distilled water, placed on moist paper in a petri dish, and incubated for 12 to 48 h to stimulate sporulation. Conidia were dislodged from the leaf tissue by rubbing using a fine glass rod and agitated in sterile distilled water. The spore suspension was spread on potato dextrose agar

Fig. 1. Map of Uganda showing the two agroecological zones where *Cercospora sorghi* and other *Cercospora* isolates were collected. Northern, moist farmland is composed of Masindi, Lira, and Apac districts, and Jinja-Mbale farmland and Lake Victoria crescent are composed of Masaka, Mpigi, Mbale, Iganga, Jinja, and Sironko districts.

(PDA) plates (Difco Laboratories, Becton Dickinson, Microbiology Systems, Sparks, MD), and cultured under light for 16 h at 22°C and in darkness for 8 h at 16°C. Monoconidial cultures of the isolates were established by hyphal tip transfer from distinct colonies on the PDA plates to fresh V8 juice agar plates (2). To obtain mycelium for DNA isolation, a culture plug of each isolate was used to inoculate 100 ml of potato dextrose broth media (Difco Laboratories). The cultures were placed on a rotary shaker and allowed to grow for 10 to 14 days under the same light and temperature regimes as described above. Mycelium was harvested by draining off excess media and freeze dried for 48 h using an

Edwards freeze dryer (Edwards High Vacuum International, West Sussex, England). Dried samples were sealed in petri dishes and stored at 4°C or directly used for DNA isolation as described (27). Isolated DNA was treated with RNaseA as described (38). DNA from two *C. zeae-maydis* sibling species, designated group I and II (42), and four other *Cercospora* spp. also were included in the study.

AFLP analysis. Genomic DNA was subjected to AFLP analysis based on fluorescent labeling and data processing as previously described (13,31). All primers used in different steps are listed in Table 2. For the selective amplification step, the *MseI*

TABLE 1. Origin and designations of the isolates used in the study

Origin	Host species	Isolates
Masindi, Uganda	Wild sorghum ^a	CSWS1, CSWS2, CSWS3, CSWS4, CSWS5
Mpigi, Uganda	Wild sorghum	CSWS6
Masaka, Uganda	Wild sorghum	CSWS7, CSWS9
Mbale, Uganda	Wild sorghum	CSWS8, CSWS16
Lira, Uganda	Wild sorghum	CSWS10, CSWS11, CSWS12
Mpigi, Uganda	Wild sorghum	CSWS13, CSWS14, CSWS15
Mbale, Uganda	Sorghum ^b	CSS1, CSS2, CSS3
Mpigi, Uganda	Sorghum	CSS4, CSS5, CSS6, CSS7, CSS8, CSS9, CSS10, CSS11, CSS12, CSS13
Masindi, Uganda	Sorghum	CSS14, CSS15
Masaka, Uganda	Sorghum	CSS16, CSS17, CSS18, CSS29
Apac, Uganda	Sorghum	CSS19, CSS20
Sironko, Uganda	Sorghum	CSS22, CSS23, CSS24, CSS25
Lira, Uganda	Sorghum	CSS26, CSS27
Apac, Uganda	Sorghum	CSS28
Illinois, USA	Maize ^{c,d}	CZM-IL-2
Illinois, USA	Maize ^{c,e}	CZM-GLS 2
Indiana, USA	Maize ^{c,d}	CZM-In-24
United States	Maize ^{c,d}	CZM-SCOH 1-5
Ohio, USA	Maize ^{d,f}	CZM-OH-8
New York, USA	Maize ^{d,f}	CZM-NY2
Mpigi, Uganda	Maize ^{e,f}	CZM-2a
Lira, Uganda	Maize ^{e,f}	CZM-Ug1
Masindi, Uganda	Maize ^{e,f}	CZM-Ug3
Iganga, Uganda	Maize ^{e,f}	CZM-Ug10
Jinja, Uganda	Maize ^{e,f}	CZM-Ug27
Kisumu, Kenya	Maize ^{e,f}	CZM-Kg20
Mpigi, Uganda	Cassava ^g	<i>Cercospora henningsi</i>
Mpigi, Uganda	Peanuts ^h	<i>C. arachidicola</i>
Mpigi, Uganda	Peanuts	<i>C. personata</i>
Germany	Sugar beet ⁱ	<i>C. beticola</i> 1, <i>C. beticola</i> 2, <i>C. beticola</i> 7
France	Sugar beet	<i>C. beticola</i> 3
Denmark	Sugar beet	<i>C. beticola</i> 4
France	Sugar beet	<i>C. beticola</i> 5
Ukraine	Sugar beet	<i>C. beticola</i> 6

^a Isolated from wild sorghum (*Sorghum bicolor* var. *verticilliflorum*).

^b Isolated from cultivated sorghum (*S. bicolor*).

^c *Cercospora zeae-maydis* group I.

^d From Wang et al. (42).

^e From Okori et al. (31).

^f *C. zeae-maydis* group II.

^g Isolated from cassava (*Manihot esculenta*).

^h Isolated from peanuts (*Arachis hypogea*).

ⁱ Isolated from sugar beet (*Beta vulgaris*).

TABLE 2. Description of the primers used for amplified fragment length polymorphism analysis

Primer	Function	Sequence
EA 1.1	<i>EcoRI</i> -adapter	5' CTCGTAGACTGCGTACC 3'
EA 1.2	<i>EcoRI</i> -adapter	5' AATTGGTACGCAGTC 3'
MA 1.1	<i>MseI</i> -adapter	5' GACGATGAGTCCTGAG 3'
MA 1.2	<i>MseI</i> -adapter	5' TACTCAGGACTCAT 3'
Pre- <i>EcoRI</i>	Preamplification	5' GACTGCGTACCAATTTC 3'
Pre- <i>MseI</i> C	Pre- and selective amplification	5' GATGAGTCCTGAGTAAC 3'
<i>EcoRI</i> AT	Selective amplification	5' AGACTGCGTACCAATTCAT 3'
<i>EcoRI</i> GA	Selective amplification	5' AGACTGCGTACCAATTCGA 3'
<i>EcoRI</i> GT	Selective amplification	5' AGACTGCGTACCAATTCGT 3'
<i>EcoRI</i> GC	Selective amplification	5' AGACTGCGTACCAATTCGC 3'
<i>EcoRI</i> AG	Selective amplification	5' AGACTGCGTACCAATTCAG 3'
<i>EcoRI</i> CC	Selective amplification	5' AGACTGCGTACCAATTCCT 3'

TABLE 3. Analysis of molecular variation based on 984 amplified fragment length polymorphism fragments of *Cercospora sorghi* isolates collected from two Ugandan agroecological zones

Source of variation	df	Variance components	Percentage of variation
Among agroecologies ^a	1	0.636	0.39
Among populations within agroecologies	4	0.402	0.25
Within populations	38	159.91	99.4

^a $\phi F_{ST} = 0.01$, $P = 0.291$. Agroecologies from which the fungal isolates were collected (i.e., Northern moist farmlands and Jinja-Mbale farm lands and Lake Victoria crescent, respectively).

TABLE 4. Gene diversity and numbers of polymorphic loci of Ugandan *Cercospora sorghi* populations based on 984 amplified fragment length polymorphism loci examined

District of origin	Number of polymorphic loci	Average gene diversity ($\bar{H}$) ^a
Lira	673	0.345
Apach	532	0.361
Masindi	767	0.345
Mbale	749	0.301
Mpigi	851	0.338
Masaka	711	0.320

^a Average gene diversity computed according to Nei (28) and Tajima (39). $\bar{H}$ values are not significantly different at $P \leq 0.05$. Tests performed according to Nei (28).

primer, Pre-*MseI* C, was used in combination with all the six *EcoRI*-selective primers, giving a total of six primer combinations. The *EcoRI* selective primers were labeled with fluorescent dyes HEX or NED at the 5' end of the primers. Each primer combination generated ≈ 100 to 180 fragments visualized as peaks. Two kinds of software, GeneScan Analysis, version 2.1, and Genotyper, version 2.0 (Perkin-Elmer/Applied Biosystems, Foster City, CA) were used to analyze peaks. GeneScan was used to generate a data matrix in the form of electrophorograms from each gel run, while Genotyper generated a binary data matrix from GeneScan files. Overlapping peaks, ambiguous peaks, and noise signals were eliminated by selecting peaks that attained a scaled height of 100 bp and by using the overlap skip function and manual editing of the data as described by the manufacturer (Perkin Elmer/Applied Biosystems). The scaled height of 100 bp was used based on experience with other fungi; that is, *C. zeae-maydis* (30) and *Verticillium* sp. (13), which showed that fragments below 100 bp were generally noise signals.

Restriction analysis of ITS and mitochondrial rDNA genes.

Genetic relatedness of Ugandan *C. sorghi* and other *Cercospora* spp. was studied by PCR-RFLP analysis of the entire ITS regions (ITS1, 5.8S rDNA and ITS2, rDNA), and the mtSSU rDNA. The ITS and mtSSU regions were amplified by PCR with the primers ITS4 and ITS5 and MS1 and MS2 for ITS and mtSSU, respectively (43), as described by Shi et al. (36). The PCR products were purified by extraction with chloroform and precipitated with sodium acetate and ethanol. Purified ITS and mtSSU PCR fragments were digested with *RsaI*, *AluI*, *TaqI*, or *MboI* (MBI Fermentas, Lithuania), separated by electrophoresis on 6% denaturing polyacrylamide gels to maximize resolution of digestion products and visualized by silver staining with the Cast Away silver staining kit (Stratagene, La Jolla, CA).

Data analysis. Each AFLP fragment was considered as a single locus with two alleles, present and absent. Data generated from each primer combination were pooled to generate one multilocus data set comprising 984 fragments that subsequently was used in genetic analyses. For population genetic analyses, the 53 fungal isolates were structured into two populations divided by their agroecological zone of origin. A null hypothesis indicating presence of a population structure among test isolates was analyzed by Nei's coefficient of population differentiation (G_{ST}) (28). Further tests for population differentiation included exact tests at the 5% significance level (35). Indirect estimates of gene flow be-

tween populations were attained by G_{ST} with $Nm = 1/2(1 - G_{ST})/G_{ST}$, (25), where N = the effective population size, m = the immigration rate, and Nm = the average number of migrants among populations per generation (37). G_{ST} , Nm , and genetic distance between the two populations were computed with PopGene (46). The data also were subjected to analysis of molecular variation (AMOVA) (12) with Arlequin version 2 (35). AMOVA partitioned total variance into hierarchical components (among populations, among subpopulations, and within subpopulations). Significance of the fixation index analogue ϕF_{ST} , computed in AMOVA, was tested using nonparametric permutation (35). The average gene diversity over multiple loci ($\bar{H}$) (i.e., the gene diversity at nucleotide level was calculated (28,39) and the $\bar{H}$ values were compared using a t test (28). AFLP data also were subjected to phenetic analysis using the neighbor-joining algorithm in TREECON, version 1.3b (41). Robustness of the phenogram was tested by bootstrap analysis of 1,000 replicates.

RESULTS

Population differentiation and phenetic analysis. Nei's coefficient of population differentiation (G_{ST}) was 0.03, leading to acceptance of the null hypothesis stating an absence of population differentiation between the two populations in Uganda. Similar results were obtained from the exact test ($P > 0.05$) and AMOVA ($\phi F_{ST} = 0.01$, $P = 0.291$) (Table 3). The results of the AMOVA analysis also revealed that the allelic variation (99.4%) was shared between populations. Average gene diversity over all loci ranged from 0.301 to 0.361, showing higher diversity within populations rather than between populations (Table 4). Gene diversity estimates between the two populations was 0.329 ± 0.137 and was not significantly different within and between populations. Nei's genetic distance between the populations was relatively small (0.014). The number of polymorphic loci were similar for the populations studied (Table 4). Indirect estimates of migration Nm were high, at an average of 20 between the two populations. Phenetic analysis revealed the presence of two major clusters (Fig. 2). One cluster is composed of *Cercospora* spp. that are pathogenic on monocot host plant species (cultivated and wild sorghum, and maize). Within this cereal cluster, there was a predominant *C. zeae-maydis* subcluster with a strong bootstrap support which also had four *C. sorghi* isolates. No major differences between isolates of *C. sorghi* collected from wild or cultivated sorghums were observed, with the exception of some minor nodes that had strong bootstrap support. The second cluster comprised *C. henningsi*, *C. arachidicola*, *C. personata*, and *C. beticola*, which are pathogenic on four dicotyledonous plant species (Fig. 2).

Restriction analysis of ITS and mitochondrial small rDNA. PCR-RFLP analysis of the *C. sorghi* ITS gave a similar pattern for most of the four restriction enzymes tested. The restricted ITS rDNA fragments varied in number from three to four, with fragment sizes ranging from 150 to 300 bp depending on the enzymes used. The digested mtSSU similarly had three fragments, with the digest fragment sizes ranging from ≈ 120 to 400 bp depending on the enzymes used. In general, for all the restriction enzymes tested, *C. sorghi* isolates were monomorphic irrespective of their host of origin, with one or two isolates being polymorphic (Fig. 3A and B). The polymorphic isolates, however,

0.1

Fig. 2. Unrooted neighbor-joining dendrogram of amplified fragment length polymorphism (AFLP) based on 984 fragments of 44 *Cercospora sorghi* (CSS and CSWS), 7 *C. zeae-maydis* (CZM), 7 *C. beticola*, and 1 each of *C. arachidicola*, *C. personata*, and *C. henningsi* isolates. Bootstrap values above 80%, generated from 1,000 replications, are indicated. A bar indicates genetic distances.

had restriction sites similar to those of other *C. sorghi* and, interestingly, *C. zea-maydis* isolates both for ITS and mtSSU. In some cases, divergent *C. sorghi* had restriction products similar to *C. zea-maydis*, for both ITS and mtSSU (Fig. 3A and B). In the case of *C. zea-maydis*, which has two known haplotypes designated group I and group II (42), the restriction enzymes *RsaI*, *HpaII*, and *AluI* gave clear diagnostic patterns permitting discrimination between the two *C. zea-maydis* haplotypes and *C. sorghi* (Fig. 3A).

DISCUSSION

In the present study, we investigated genetic variability of *C. sorghi* in Uganda from cultivated and wild sorghum plants to gain insight into its evolutionary history and potential to evolve. On the basis of population structure, gene flow, and presence or absence of sexual phases, the potential evolutionary risk associated with fungal plant pathogens can be predicted (25). Determination of genetic or population structures provides insights into the degree of genetic diversity of a pathogen or any other organism and lays a foundation for its population genetic characterization. In this study, estimates of Wright's population differentiation index G_{ST} and ϕF_{ST} values ranged from 0 to 0.05. In other fungal pathosystems, population differentiation indices within this range have been interpreted as indicative of low genetic differentiation between populations (16,21,22). A low genetic differentiation suggests a close genetic relatedness of the populations. In the case of *C. sorghi*, this would imply that the pathosystem is, in fact, one

epidemiological unit with most of the observed allelic variation shared among populations from cultivated and wild sorghums. The presence of a population structure is indicative of high genetic diversity largely due to genetically separated populations, which is the not the case with *C. sorghi*. The small genetic distance between isolates from the two agroecological zones further supports our suggestion of one *C. sorghi* epidemiological unit. AMOVA revealed that up to 99% of the allelic variation was shared among populations. We did not find strong evidence that such variability could be attributed to differences in host species infected by *C. sorghi* as has been reported for *Tilletia* spp. (33), *Phytophthora infestans* (14), and *Magnaporthe grisea* (4). One possible explanation for this discrepancy could be the role of a sexual phase and extensive selection pressure at the host level in the case of those other fungal pathogens, compared with the absent sexual stage in *C. sorghi* and limited breeding for resistance against gray leaf spot in sorghum. Therefore, because most *Cercospora* spp. have a very restricted host range (7), it seems logical to assume that *S. bicolor* var. *verticilliflorum*, the progenitor of cultivated sorghum, may indeed play a role in gray leaf spot epiphytotics. The *C. sorghi* phenetic tree shows some degree of allelic variability among the isolates, as indicated by the presence of minor groups within the main group. Thus, the observed allelic variability could be attributed to maintenance of various *C. sorghi* clones within the population as a result of low selection pressure. Both sorghum species share agroecologies and, once harvested, the wild sorghum would perhaps act as an inoculum bank in the next cropping season, introducing novel *C. sorghi* into cropping fields. PCR-RFLP analysis of ITS and mtSSU showed, in general, a monomorphic profile for all isolates collected from the wild or cultivated sorghum, although some isolates clearly were distinct from the rest. The distinct isolates shared restriction sites with both *C. sorghi* and *C. zea-maydis*, alluding to variability among isolates but indicating that they are indeed *C. sorghi*. Identity of the divergent *C. sorghi* also was tested by sequencing the ITS and cytochrome *b* gene of randomly selected *C. sorghi* and the divergent isolates from the phenetic and PCR-RFLP analyses. Sequence alignments, including downloaded *C. sorghi* ITS rDNA sequences, showed that the divergent isolates were indeed *C. sorghi* and not *C. sorghi* var. *maydis*, a variant of *C. sorghi* that cross-infects maize, causing gray leaf spot (7) (data not shown). Recent genomic studies of *Cercospora* and *Mycosphaerella* fungi show that, within the genus *Cercospora*, up to six nucleotide differences may be observed in the ITS region (18). Such nucleotide differences change restriction sites and this may account for the variation observed in PCR-RFLP analysis. Additionally, the identity of ITS and mtSSU restriction digest profiles suggests that these *C. sorghi* isolates may have originated from a common gene pool. Gene flow has been implicated in other fungal pathosystems as an opposing evolutionary force to genetic differentiation between fungal populations (14,21,22,26). Exchange of one individual per generation generally is considered sufficient to prevent substantial fixation of alleles in a population (25,37,45). In this study, *N_m* had an estimated value of 20, suggesting extensive gene flow between populations. Population studies of *Setosphaeria turcica* (anamorph = *Exserohilum turcicum* (Pass.) K. J. Leonard & E. G. Suggs), another maize pathogen causing turcicum leaf blight, revealed similar results (*N_m* = 17) for populations within Kenya and indicative of extensive gene flow within the country (3). The exact role of wild sorghum plants in gene flow between sorghum populations is not yet known. Gray leaf spot of sorghum is a late-season disease and, given that diseased wild sorghum is commonly found in sorghum fields in sub-Saharan Africa, this alternative host could be the source of inoculum and conduit for gene flow. For disease control, our data suggest that the high severity of gray leaf spot observed in some areas may be due to the effect of inoculum level, perhaps as impacted by early incursion from wild sorghum in fields.

Fig. 3. A, Restriction fragment length polymorphisms of the internal transcribed spacer region of *Cercospora sorghi* isolates CSW1 to CSW16 and CSS1 to CSS28 from *Sorghum bicolor* var. *verticilliflorum* and *S. bicolor*, respectively, and *C. zea-maydis* group I (GpI) and group II (GpII), digested with *RsaI*. **B,** Restriction fragment length polymorphisms of mitochondrial small sub-unit rDNA of *C. sorghi* isolates CSW1 to CSW15 and CSS7 to CSS29 from *S. bicolor* var. *verticilliflorum* and *S. bicolor*, respectively, and *C. zea-maydis* group I (GpI) and group II (GpII), digested with *AluI*.

The genetic structures of *C. sorghi* are similar to those we have found previously for East African *C. zea-maydis* populations in which no genetic structures was detected (31). The absence of genetic structures alludes to genetic similarity and is common among young populations and those where gene flow is common. Thus, the absence of genetic structures in these two pathogens supports the view that speciation of *C. zea-maydis* and *C. sorghi* may be a recent event (10,18). It also suggests that these pathogens may have been subjected to similar evolutionary forces and, therefore, may react in the same evolutionary manner. Comparative analysis showed that cereal and dicot plant-pathogenic *Cercospora* spp. clustered separately, with five *C. sorghi* isolates clustering with *C. zea-maydis* isolates. The two fungi are closely related and such subclustering is not uncommon (18). A similar finding was reported by Goodwin et al. (18), supporting the view that host specificity has played a crucial role in speciation within the genus. Therefore, it is interesting to find that *C. sorghi* isolates from cultivated and wild sorghums were not separated genetically. The absence of a population structure among Ugandan *C. sorghi* from a management point of view suggests, further, that the evolutionary responsiveness of this pathogen to selective disease control strategies may be slow. Genetic similarity between *C. sorghi* isolates from wild and cultivated sorghum indicates that wild sorghum may play an epidemiological role in gray leaf spot of sorghum epidemics. Taken together, our results imply that, for long-term disease management, host disease resistance could form a core component of disease management due to low evolutionary responsiveness of *C. sorghi*. Spatial characterization of gray leaf spot epidemics could elucidate disease carryover and, more specifically, the role of wild relatives in disease epidemics. This information could help improve disease control in the tropics and elsewhere.

ACKNOWLEDGMENTS

This study was funded by the SIDA-SAREC East African Biotechnology Network (BIO-EARN) project number 771799-771702. Additional funding was obtained from Nilson-Ehle Foundation. We thank L. D. Dunkle, U.S. Department of Agriculture, Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana and M. L. Clements, Crop Sciences Department University of Illinois for the U.S. *C. zea-maydis* isolates; and M. Nihlgård, Syngenta, Landskrona, Sweden, for the *C. beticola* isolates.

LITERATURE CITED

- Beckman, P. M., and Payne, G. A. 1982. External growth, penetration and development of *Cercospora zea-maydis* in corn leaves. *Phytopathology* 72:810-815.
- Beckman, P. M., and Payne, G. A. 1983. Cultural techniques and conditions influencing growth and sporulation of *Cercospora zea-maydis* and lesion development in corn. *Phytopathology* 73:286-289.
- Borchardt, D. S., Welz, H. G., and Geiger, H. H. 1997. Genetic structure of *Setosphaeria turcica* populations in tropical and temperate climates. *Phytopathology* 88:322-329.
- Borromeo, E. R., Nelson, R. J., Bonman, J. M., and Leung, H. 1992. Genetic differentiation among isolates of *Pyricularia* infecting rice weed hosts. *Phytopathology* 83:393-399.
- Burdon, J. J. 1993. The structure of pathogen populations in natural plant communities. *Annu. Rev. Phytopathol.* 31:305-323.
- Carson, M. L., Goodman, M. M., and Williamson, S. M. 2002. Variation in aggressiveness among isolates of *Cercospora* from maize as a potential cause of genotype-environment interaction in gray leaf spot trials. *Plant Dis.* 86:1089-1093.
- Chupp, C. 1953. A monograph of the fungus genus *Cercospora*. Ronald Press Co., Ithaca, NY.
- Doggett, H., and Prasada Rao, K. E. 1995. Sorghum. Pages 173-180 in: *Evolution of Crop Plants*. Longman, England, UK.
- Duncan, R. R., and De Millano, W. A. J. 1995. Plant disease control in sorghum and pearl millet. Pages 35-71 in: *Disease Analysis through Genetics and Biotechnology*. J. F. Leslie and R. A. Frederiksen, eds. Iowa State University Press, Ames.
- Dunkle, L. D., and Levy, M. 2000. The genetic relatedness of African and United States populations of *Cercospora zea-maydis*. *Phytopathology* 90:486-490.
- Emechibe, A. M. 1975. Some Aspects of Crop Diseases in Uganda. Makerere University Press, Kampala, Uganda.
- Excoffier, L., Smouse, P., and Quattro, J. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial restriction data. *Genetics* 131:479-491.
- Fahleson, J., Lagercrantz, U., Steventon, L. A., and Dixelius, C. 2003. Evaluation of genetic variation among *Verticillium* isolates using AFLP analysis. *Eur. J. Plant Pathol.* 109:361-371.
- Flier, W. G., Grünwald, N. J., Kroon, L. P. N. M., Sturbaum, A. K., van den Bosch, T. B. M., Garay-Serrano, E., Lozoya-Saldaña, H., Fry, W. E., and Turkensteen, L. J. 2003. The population structure of *Phytophthora infestans* from the Toluca valley of central Mexico suggests genetic differentiation between populations of cultivated potato and wild *Solanum* spp. *Phytopathology* 93:382-390.
- Frederiksen, R. A., Thomas, M. D., Bandyopadhyay, R., and Mughogho, L. K. 1995. Variable pathogens of sorghum. Pages 11-23 in: *Disease Analysis through Genetics and Biotechnology: Interdisciplinary Bridges to Improved Sorghum and Millet Crops*. J. F. Leslie and R. A. Frederiksen, eds. Iowa State University Press, Ames.
- Gagné, P., Yang, D.-Q., Hamelin, R. C., and Bernier, L. 2001. Genetic variability of Canadian populations of the Sapstain fungus *Ophiostoma piceae*. *Phytopathology* 91:369-376.
- Garrett, K. A., and Mundt, C. C. 1999. Epidemiology in mixed host populations. *Phytopathology* 89:984-990.
- Goodwin, S. B., Dunkle, L. D., and Zismann, V. L. 2001. Phylogenetic analysis of *Cercospora* and *Mycosphaerella* based on the internal transcribed spacer region of the ribosomal DNA. *Phytopathology* 91:648-658.
- Hankook, J. F. 1992. *Plant Evolution and the Origin of Crop Species*. Prentice Hall, UK.
- Jameson, J. D. 1970. *Agriculture in Uganda*. Oxford University Press, UK.
- Keller, S. M., Wolfe, M. S., McDermont, J. M., and McDonald, B. A. 1997. High genetic similarity among populations of *Phaeosphaeria nodorum* across wheat cultivars and regions in Switzerland. *Phytopathology* 87:1134-1139.
- Linde, C., Zhan, J., and McDonald, B. A. 2002. Population structure of *Mycosphaerella graminicola* from lesions to continents. *Phytopathology* 92:946-955.
- Marley, P. S., Elemo, K. A., Aba, D. A., Onu, I., and Akintayo, I. 2001. Reactions of sorghum genotypes to anthracnose and grey leaf spot under Sudan and Sahel savannah field conditions of Nigeria. *J. Sustain. Agric.* 18:105-116.
- Mbwaga, A. M., Pande, S., De Milliano, W. A. J., and Karunakar, R. I. 1993. Diseases and parasitic weeds of sorghum in Tanzania: Occurrence and incidence, 1986-1990. *Crop Prot.* 12:183-188.
- McDermont, J. M., and McDonald, B. A. 1993. Gene flow in plant pathosystems. *Annu. Rev. Phytopathol.* 31:353-373.
- McDonald, B. A., and Linde, C. 2002. Pathogen population genetics, evolutionary potential and durable resistance. *Annu. Rev. Phytopathol.* 40:349-379.
- Möller, E. E., Bahnweg, G., Sandermann, H., and Geiger, H. H. 1992. A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies and infected plant material. *Nucleic Acids Res.* 20:6115-6116.
- Nei, M. 1987. *Molecular Evolutionary Genetics*. Columbia University Press, New York.
- Odyssey, G. N. 1986. Gray leaf spot. Pages 11-12 in: *Compendium of Sorghum Diseases*. R. A. Frederiksen, ed. The American Phytopathological Society, St. Paul, MN.
- Okori, P., Fahleson, J., and Dixelius, C. 2001. Occurrence of gray leaf spot disease of maize in East Africa. *Fungal Genet. Newsl.* 48 (Suppl):62.
- Okori, P., Fahleson, J., Rubaihayo, P. R., Adipala, E., and Dixelius, C. 2003. Assessment of genetic variation among east African *Cercospora zea-maydis* populations using AFLP and RFLP. *Afr. Crop Sci. J.* 11:75-86.
- Pande, S., Hari Krishnan, R., Alegbejo, M. D., Mughogho, L. K., Karunakar, R. I., and Ajayi, O. 1993. Prevalence of sorghum diseases in Nigeria. *Int. J. Pest Manage.* 39:297-303.
- Pimentel, G., Peever, T. L., and Carris, L. M. 2000. Genetic variation among natural populations of *Tilletia contraversa* and *T. bromi*. *Phytopathology* 90:376-383.
- Rupe, J. C., Siegel, M. R., and Hartman, J. R. 1982. Influence of environment and plant maturity on gray leaf spot on corn caused by *Cercospora zea-maydis*. *Phytopathology* 72:1587-1591.
- Schneider, S., Roessli, D., and Excoffier, L. 2000. Arlequin ver 2.000: A software for population genetics data analysis. *Genetics and Biometry Laboratory, University of Geneva, Switzerland*.

36. Shi, Y. L., Loomis, P., Christian, D., Carris, L. M., and Leung, H. 1996. Analysis of the genetic relationships among the wheat bunt fungi using RAPD and ribosomal DNA markers. *Phytopathology* 86:311-318.
37. Slatkin, M., and Barton, N. H. 1989. A comparison of three indirect methods for estimating average levels of gene flow. *Evolution* 43:1349-1368.
38. Steventon, L. A., Fahleson, J., Hu, Q., and Dixelius, C. 2002. Identification of the causal agent of *Verticillium* wilt of winter oilseed rape in Sweden as *V. longisporum*. *Mycol. Res.* 106:570-578.
39. Tajima, F. 1983. Evolutionary relationship of DNA sequences in finite populations. *Genetics* 105:435-460.
40. Thomas, M. D. 1991. Development of gray leaf spot on sorghum in Burkina Faso. *Plant Dis.* 75:45-47.
41. Van de Peer, Y., and De Watcher, R. 1994. TREECON for windows. A software for the construction and drawing of evolutionary trees for Microsoft Windows environment. *Comput. Appl. Biosci.* 10:569-570.
42. Wang, J., Levy, M., and Dunkle, L. D. 1998. Sibling species of *Cercospora* associated with gray leaf spot of maize. *Phytopathology* 88:1269-1275.
43. White, T. J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 316-322 in: *PCR Protocols: A Guide to Methods and Applications* A. I. Michael, D. H. Gelfand, J. J. Sninsky, and T. J. White, eds. Academic Press, New York.
44. Wortmann, C. S., and Eledu, C. A. 1999. Uganda's Agroecological Zones: A Guide for Planners and Policy Makers. Centro Internacional de Agricultura Tropical, Kampala, Uganda.
45. Wright, S. 1951. The genetic structure of populations. *Ann. Eugen.* 15:323-354.
46. Yeh, F. C., and Boyle, T. J. B. 1997. Population genetic analysis of co-dominant and dominant markers and quantitative traits. *Belg. J. Bot.* 129:157.