

MYCOFLORAL DIVERSITY AND MOLECULAR CHARACTERIZATION OF SPECIES ISOLATED FROM FARMER- SAVED RICE SEEDS IN THE IRRIGATED RICE PRODUCTION DISTRICTS OF THE COASTAL SAVANNAH ZONES OF GHANA

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ABSTRACT

Irrigated rice production is the major type of rice production in the Coastal Savannah Zone of Ghana, where farmers rely on their saved seeds for production. A study was carried out to determine the types of storage fungi resident on farmer saved seeds and their distribution in five major rice production areas of the Coastal Savannah Zone. The blotter method was used to isolate fungal species after which they were identified using cultural and morphological features complemented by sequence analysis of the entire Internal Transcribed Spacer (ITS1-ITS2) region of isolates. Eleven fungal species namely, *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Colletotrichum gloeosporioides*, *Curvularia lunata*, *Curvularia geniculata*, *Fusarium equiseti*, *Lasiodiplodia theobromae*, *Rhizopus oryzae* and *Trichoderma* sp. belonging to 8 different genera were isolated and identified on the rice seeds. *Curvularia lunata*, with a percentage occurrence of 63.9% was the most prevalent fungal species, while *Trichoderma* sp. (1.3%) was the least prevalent fungal species from the study area. The high infection rate of seeds by *Curvularia* species may lead to high incidence and severity of *Curvularia* leaf spot disease in the study area.

Keywords: Resident mycoflora, irrigated rice seeds, conventional and molecular characterisation of fungi, Coastal Savanna Zone, Ghana.

Introduction

Rice (*Oryza sativa* L.) is an important source of calories for most Ghanaians. Reports show that there is gradual shift from the consumption of other staple foods such as maize and cassava, to the consumption of rice in Ghana. This has been attributed to urbanisation and change in people's taste. Consequently, there

have been strategies to either increase the local production of the crop or find more money to increase imports. According to Alhassan *et al.* (2015) there has been a gradual increase in rice consumption levels in Ghana from 790,000 MT to around 926,000 MT from 2010 to 2015 respectively. Due to the short supply of rice to meet consumer demand in Ghana, the country

relies on imported rice. A total of 4.5 million dollars was spent on rice imports into the country by the year 2015 (GNA, 2015).

In Ghana, rice production is concentrated around the Northern and Coastal parts of the country. In the Northern parts, production is dominated by the rain-fed lowland ecology, while in the coastal savanna parts of the country, irrigated rice production ecology dominates (Bidzakin *et al.*, 2018). Irrigated rice ecology is reported to have the highest rice yields because of the level of technology utilization compared to rain fed lowland and upland ecological topographies (Bidzakin *et al.*, 2018). Irrigated rice production is common in areas such as Afiénya in the Greater Accra region, Afife in the Volta region and Akuse and Kpong in the Eastern region. In these areas, dams are available making it possible to have all year-round irrigation for the production of rice.

Most rice farmers in Ghana cultivate their fields using farmer-saved seeds. Repeated use of farmer-saved seeds comes with detrimental consequences. For example, this can compromise the health status of the seeds. This is primarily because farmers' storage conditions are not ideal which consequently lead to their contamination by seed-borne pathogens (Nutsugah *et al.*, 2004). The use of poorly stored seeds by farmers predisposes the seeds to infection by disease causing fungi and this adversely affects viability of seeds. Consequently, rice establishment both at the nursery and in the field are adversely affected leading to a decline in the overall production level of farmers (Mew *et al.*, 1994; Horna *et al.*, 2006; Shetty, 2010). Seed-borne microorganisms such as fungi, bacteria, nematodes and viruses are responsible for transmitting seed-borne diseases, which often

cause reduction in overall crop yields (Barret, 2015). When all aspects of seed quality are achieved, farmers have greater chances of good harvest (Miva *et al.*, 2017).

In the irrigated rice production areas of the Coastal savanna zone of Ghana, few studies have shown that the farmer saved-seeds are contaminated with some fungal species such as *Curvulaia lunata*, *Fusarium* sp and *Alternaria lunata* (Osumanu, 2012). Current observations have shown increases in foliar diseases of rice in the fields. It has been suggested that this could be as a result of seed recycling, which may be contaminated with seed transmissible fungal species (Honger, unpublished data). It has been suggested therefore, that, the quality of seeds being used by farmers in these areas need to be re-assessed, to determine the extent of contamination by fungal pathogens.

Fungal identification in rice seeds and other products in Ghana has been carried out using cultural, morphological, colour and growth characterisation. In some instances, this can lead to misidentification especially when dealing with closely related species in the same genus. Environmental and cultural media composition may influence fructification and cultural morphology in some instances (Phillips *et al.*, 2008). On the other hand, molecular characteristics methods are more exact based on genetic inborn markers and are not usually influenced by environmental conditions. In view of this, a combination of conventional methods and molecular methods have been the current trend for authentication of fungi, especially where facilities are available such as in cases of *Collectotrichum gleosporiodes* on mango (Honger *et al.*, 2014) and on cocoa (Amoako-Attah *et al.*, 2020). It is therefore imperative that this strategy is adopted to properly identify fungal species

infecting rice seeds, since the formulation of a good control measure against an organism is dependent on its accurate identification.

The entire internal transcribed spacer region of fungi has been used as barcode for species delineation and found to be very accurate and reliable (Weir *et al.*, 2012) and can be used solely to distinguish among different fungi, especially when they are not closely related (White *et al.*, 1990). In this paper, we have combined both the conventional and molecular methods of sequence analysis of the entire ITS region of the isolated fungi to ascertain the diversity of fungi associates with farmer-saved rice seeds in the irrigated rice growing districts of the Coastal Savannah Zone of Ghana.

Experimental

Experimental sites

Farmer-saved rice seeds were collected from 75 individual rice farmers in five Districts of the Coastal Savanna Zone of Ghana (Table 1). One major irrigated rice production area per District was specifically selected and 15 farmers were randomly selected, with the help of Agricultural Extension Officers. Exactly 1.5 kg rice seeds were collected from each farmer, labeled and sent to the Plant Pathology Laboratory of the Department of Crop Science, University of Ghana, for the seed health test.

TABLE 1

Locations in the coastal savannah zone of Ghana where farmer-saved rice seeds samples were collected for the study

Location	District/Municipal	Administrative Region
Akuse	Lower Manya Krobo	Eastern
Afife	Ketu North	Volta
Ashiaman	Ashiaman	Greater Accra
Asutuare	Shai-Osudoku	Greater Accra
Kpong	Lower Manya Krobo	Eastern

Seed health test

This was carried out to distinguish resident fungal pathogens from surface contaminants fungi. The samples were analyzed using the Blotter Method (ISTA, 1999).

Blotter test method

Four hundred (400) seeds, randomly sampled from each seed sample, were sterilized using 1% sodium hypochlorite (NaOCl) for 60 seconds and rinsed in three changes of distilled water and dabbed dried with a sterile tissue paper. Two hundred (200) seeds were sub sampled and 25 were placed in each of 8 petri dishes containing moist filter paper. Petri plates were labelled appropriately and then coded. Plated seeds were incubated at $22 \pm 2^\circ\text{C}$ under 12 hours alternate cycle of ultra-violet light and darkness for 7 days. Seeds were first examined after incubation under the stereomicroscope and bits of mycelia of fungi that were observed, were taken with an inoculation pin and plated on PDA (2 g/l). Between 5 and 7 days, when enough growth was observed on the PDA, the isolates were identified using their cultural and morphological features complemented with molecular methods (polymerase chain reaction and BLAST search).

Cultural and morphological identification of isolates

The nature of growth and colour of the mycelium of isolates and number of days taken by mycelium to cover the entire 9 mm Petri dish, were recorded to aid in the identification of isolates. After that, bits of the mycelia and few spores were fixed on a microscope slide and the preparation observed under the compound microscope using the x40 lens. The nature of the hyphae and spores were recorded to further aid in the identification of the isolates, with the aid of published materials.

Polymerase chain reaction

Thirteen isolates of different morphological features were selected for molecular characterization. These isolates were designated with arbitrarily selected codes (Table 2). Deoxyribonucleic acid (DNA) was extracted from isolates using the CTAB (Cetyl trimethylammonium bromide) based method (www.zymoresearch.com). Isolates were cultured on PDA for 6 days prior to the DNA extraction. Polymerase chain reaction (PCR) was carried out with the DNA extracted from isolates as templates. The primer pair ITS1/ITS4, (ITS1: 5'-TCCGTAGGTGAACCTGCGG-3' and ITS4: 5'-TCCTCCGCTTATTGATATGC-3') designed to amplify the entire Internal Transcribed Spacer (ITS) region of isolates (White et al. 1990) was used in the PCR. The mixture was made up of 5 µl of template DNA, 2.5 µL each of forward and reverse primers, 1.25 µl of 2 mM MgCl₂, 25 µl of master mix (10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM dNTPs, 5% Glycerol, 0.08% IGEPAL® CA-630, 0.05% Tween® 20, 25 units/ml Taq DNA Polymerase, pH 8.6@25°C) (New England Biolabs, UK) and 13.75 µl of deionized autoclaved water. A negative control was included in the amplification. The PCR cycles were as follows: initial denaturation at 95 °C for 30 s, followed by 35 cycles of denaturing, annealing and extension at 95 °C for 10 s, 59 °C for 15 s and 72 °C for 30 s, with a final extension at 72 °C for 5 min. The Amplification products were separated by 1.5% w/v agarose gel (Invitrogen, Carlsbad, CA), stained with Ethidium bromide alongside 1.0 kb marker at 100 V for about 1.5 hours. Bands were observed under UV light.

Purification and sequencing of amplified products

The amplified PCR products were sent to Inqaba Biotech in South Africa for purification and sequencing. Ten picomole of each primer was used to sequence the product directly from both directions. Obtained sequences were manually edited and consensus strand for each isolate generated from the forward and reverse strands using the BIOEDIT software. The assembled sequences were deposited in the Genbank.

BLAST search

Consensus strands of isolates were used in a Basic Local Alignment Search Tool (BLAST) (www.blast.ncbi.nlm) search and the most similar isolate in terms of the nucleotide sequences selected as the species of the isolates obtained in this study. The expected value (E-value) which indicates the probability of finding a correct match by chance was recorded together with the percentage similarity. Hits (sequences in the data base that matched the query or yet to be identified sequence) with E values less than 10⁻⁴ is considered very significant.

Frequency of occurrence of fungal species

The number of farmers saved-seeds samples, in which a particular fungus was found was used to calculate percentage occurrence of that particular fungus per location using the formula:

$$\text{Percentage occurrence} = \frac{\text{number of samples containing the fungus} \times 100}{\text{Total number of samples}}$$

The calculated percentages were used to describe the distribution of the fungal species in the farmer-saved rice seeds samples in the

study area. The mean percentage occurrence of rice seeds by each fungus was calculated by averaging the percentage occurrence from the five districts. This was used to determine the most frequently and least occurring fungi in the study area.

Percentage infection of farmer saved-seeds

An infected rice seed sample was a sample in which at least, one type of fungi was detected on the seeds after the blotter test. To determine the extent of contamination of farmer-saved rice seeds, the number of infected seed samples from each locality was determined and used to calculate percentage infection, using the formula:

$$\text{Percentage occurrence} = \frac{\text{number of infected seed samples}}{\text{Total number of samples examined}} \times 100$$

Results

Identification of fungal species

Eleven fungal species from 8 different genera were isolated and identified from farmer saved seeds, based on the cultural and morphological features and BLAST search. These were; *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Colletotrichum gloeosporioides*, *Curvularia lunata*, *Curvularia geniculata*, *Fusarium equiseti.*, *Lasiodiplodia theobromae*, *Rhizopus oryzae* and *Trichoderma* sp. The cultural and morphological features of all isolates obtained in this study, particularly, the nature of mycelial growth on PDA, spore curvature/the nature of conidial heads (Fig.1) were consistent with what had been reported (Agrios, 2005; Barnett & Hunter, 2003; Mathur & Kongsdal, 2003) and were as follows:

Cultural and morphological features of isolated fungi

Isolates of *A. alternata* produced black to olivaceous-black mycelia which took 7 days to fill a 9 mm Petri dish (Fig. 1A). Mycelium was made up of hyphae that were cylindrical, erect and arose singly. Conidia were large and dark in colour with short beaks and were septated (Fig. 1B).

Colonies of *A. flavus* produced olive to lime green colouration with a cream reverse on potato dextrose agar. The mycelium texture was woolly (Fig. 1C). Hyphae were septated and hyaline. Conidial heads were radiate to loosely columnar with age (Fig. 1D). Conidiophores were coarsely roughened and hyaline.

Aspergillus fumigatus isolates produced smoky grey-green mycelia with a slight yellow reverse on PDA (Fig. 1D). Very mature colonies turned grey. Texture of the mycelium was woolly. Hyphae were septate and hyaline. Conidial heads were strongly columnar (Fig. 1E). Conidia were smooth to finely roughened and sub globose.

Colonies of *A. niger* produced mycelia which was initially white which changed to black as the culture aged (Fig. 1F). The reverse was pale yellow. Under the microscope, the conidial heads were radiate with conidiogenous cells being biseriate (Fig. 1G). Conidia were many and brown to black in colour.

Isolates of *C. gloeosporioides* produced white mycelium which grew to fill the entire 9 mm Petri dish in 8 days (Fig. 1J). Conidia were short rods and rounded at the edges (Fig. 1K). The conidia were produced abundantly in disc shaped acervuli produced in concentric rings in the medium.

Curvularia lunata isolates produced suede-like to downy, black colonies of mycelium which took 8 days to fill an entire 9 mm Petri dish containing PDA (Fig. 1L). The hyphae were septate and dark in colour. The conidiophore was erect, septate, unbranched and dark reddish brown in colour. The conidia were ellipsoidal, smooth-walled, rounded at the end or become thinner toward the end. Its olive brown end cells are paler while it is large, dark and curved at the subterminal cell. The subterminal cell bulge and is larger than the rest of the cells (Fig. 1M).

The isolates of *C. geniculata* produced an expanding, black and hairy mycelium on PDA (Fig. 1P). Under the microscope, the conidiophores were erect, unbranched, septate and flexuose in the apical part, with flat, dark brown scars. Conidia are smooth-walled, dark brown, 4-septate with the central cell being the largest (Fig. 1Q).

Fusarium equiseti isolates produced extensive and cotton-like in mycelium on PDA (Fig. 1R) A). Conidiophores were slender and simple, short, branched regularly or bearing a whorl of phialides. Two kinds of conidia, micro and macro were present. Microconidia are 1-celled, ovoid and were borne singly or in chains. Macroconidia are several celled, slightly curved or bent at the pointed ends, typically canoe shaped (Fig. 1S). Both types of conidia were hyaline.

Isolates of the *L. theobromae* produced a thin mycelium which covers the entire 9 mm plate in three days. The mycelium grew fluffy in 5 days (Fig. 1T). The colour of the mycelium was initially white but changed to grey and finally black within 7 days. The hyphae were initially hyaline and non-septated. The conidia were initially unicellular subovoid to ellipsoidal in shape. At maturity, conidia were

bi-celled, thick walled and ellipsoidal in shape (Fig. 1U).

Rhizopus oryzae produced grey mycelium on PDA (Fig. 1V). Hyphae were filamentous and branching, stolons, rhizoids and sporangiophores were observed (1W). Black coloured sporangia were produced abundantly in the medium.

Isolates of *Trichoderma* sp. produced colony forms that are circular as rings on PDA (Fig. 1X). The colony is light green in colour with oval conidia forms. Conidiophores were highly branched. Typically, the conidiophore terminates in one or a few phialides. Main branches of the conidiophores produce lateral side branches that were paired, the longest branches distant from the tip.

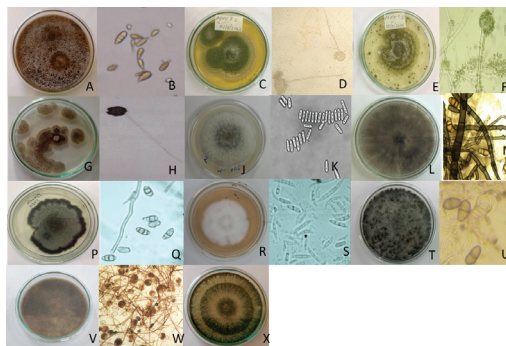


Fig.1. Cultural and morphological characteristics of fungal species isolated from farmer saved rice seeds on PDA. A and B=mycelial growth and spores of *A. alternata*, C and D=mycelial growth and conidial head of *A. flavus*, E and F= mycelial growth and conidial head of *A. fumigatus*, G and H=mycelial growth and conidial head of *A. niger*, J and K=mycelial growth and spores of *C. gloeosporioides*, L and M=mycelial growth and spores of *C. lunata*, P and Q=mycelial growth and spores of *C. geniculata*, R and S=mycelial growth and spores of *F. equiseti*, T and U=mycelial growth and spores of *L. theobromae*, V and W=mycelial growth and conidial heads of *R. oryzae* and X=mycelial growth of *Trichoderma* sp. MG=X400

BLAST search

An approximately 600 PCR products of the entire ITS region of isolates used in the experiment, was amplified. The sequenced and assembled nucleotides were approximately 550 bp and have been deposited in the GenBank (<https://www.ncbi.nlm.nih.gov>) with

accession numbers, MW600259- MW600271. Blast search resulted in the identification of all isolates to the species level (Table 1). Almost all isolates were 100% similar to the documented isolates in the GenBank, with high E values of 0.0 each.

TABLE 2

Isolate designation and cultural and morphological identification and blast search of fungal species isolated from farmers saved rice seeds

Isolate Designation (Code)	Cultural/ Morphological Identification	Genbank Accession Number	Most Identical Isolate in Genbank	Percent Similarity	E Value
CLCRS-01	<i>Curvularia sp.</i>	MW600259	<i>C. lunata</i>	100.0	0.0
CLCRS-02	<i>Curvularia sp.</i>	MW600260	<i>C. lunata</i>	100.0	0.0
CLCRS-03	<i>Curvularia sp.</i>	MW600261	<i>C. lunata</i>	100.0	0.0
CLCRS-04	<i>Curvularia sp.</i>	MW600262	<i>C. lunata</i>	100.0	0.0
CLTRS-05	<i>L. theobromae</i>	MW600263	<i>L. theobromae</i>	100.0	0.0
CANRS-06	<i>Aspergillus niger</i>	MW600264	<i>A. niger</i>	100.0	0.0
CCGRS-07	<i>Curvularia sp</i>	MW600265	<i>C. geniculata</i>	100.0	0.0
CAFERS-08	<i>Aspergillus sp.</i>	MW600266	<i>A. fumigatus</i>	100.0	0.0
CFERS-09	<i>Fusarium sp.</i>	MW600267	<i>F. equiseti</i>	100.0	0.0
CRORS-010	<i>Rhizopus sp.</i>	MW600268	<i>R. oryzae</i>	100.0	0.0
CAFL-011	<i>Aspergillus flavus</i>	MW600269	<i>A. flavus</i>	100.0	0.0
CAARS-012	<i>Alternaria alternata</i>	MW600270	<i>A. alternata</i>	100.0	0.0
CCGLRS-013	<i>C. gloeosporioides</i>	MW600271	<i>C. gloeosporioides</i>	100.0	0.0

Fungal species diversity in the five sampling localities

All the fungal species isolated in the samples from the rice irrigation districts were found in different proportions (%) (Table 3). The fungal species isolated in each sampling location can be summarized as follows in decreasing order, based on their percentage occurrences:

Afife

Curvularia lunata (93.3%) < *R. oryzae* = *A. flavus* (80%) < *Fusarium equiseti* (66.7%) < *A. fumigatus* (53.3%) < *Alternaria alternata* (33.5%) < *L. theobromae* (26.7) (Table 3).

Akuse

A. Flavus (66.7%) < *F. equiseti* (50%) < *C. lunata* = *R. oryzae* (53.3%) < *C. geniculata* (33.3%) = *C. gloeosporioides* < *L. theobromae* (13.3%) = *A. niger* (13.3%) = *A. fumigatus* (Table 3).

Ashaiman

A. flavus (80%) = *F. equiseti* = *R. oryzae* < *C. lunata* (60%) < *A. fumigatus* (46.7%) < *A. alternata* (26.7%) = *C. gloeosporioides* < *A. niger* (20%) = *C. geniculata* < *Trichoderma sp.* (6.7%) (Table 3).

Asutsuare

F. equiseti (66.7%) < *C. gloeosporioides* (60%) = *A. flavus* < *C. lunata* (53.3%) < *A. fumigatus* (33.3%) = *A. niger* = *L. theobromae* = *R. oryzae* (Table 3).

Kpong

C. lunata (86.7%) < *C. geniculata* (66.7%) < *F. equiseti* (53.3%) < *C. gloeosporioides* (46.7%) < *A. flavus* (40%) < *A. alternata* (38.3%) = *R. Oryzae* < *A. niger* < *A. fumigatus* (13.3%) = *L. theobromae* (Table 3).

Generally, *Trichoderma* sp was the least frequently encountered fungus ranging from nil (Afife, Akuse, Asutsuare, Kpong) to 6.7% (Ashaiman) (Table 3). The most predominant fungi isolated varied from one location to another and was not uniform. For example, *C. lunata* was highest at Afife (93.3%) and Kpong (86.7%); *A. flavus* was also predominant at Afife and Ashaiman (80%) while *F. equiseti* was highest at Ashaiman (80%) and *R. oryzae* predominated at Afife and Ashaiman (80%).

TABLE 3

Occurrence of fungal species obtained from farmer saved seeds in the irrigated rice ecology of the Coastal savannah zone of Ghana

Fungal Species	Locations/Percentage Occurrence (%)				
	Afife	Akuse	Ashiaman	Asutsuare	Kpong
<i>Alternaria alternata</i>	33.3	20.0	26.7	46.0	33.3
<i>Aspergillus flavus</i>	80.0	66.7	80.0	60.0	40.0
<i>Aspergillus fumigatus</i>	53.3	13.3	46.7	33.3	13.3
<i>Aspergillus niger</i>	13.3	13.3	20.0	33.3	20.0
<i>Curvularia lunata</i>	93.3	53.3	60.0	53.3	86.7
<i>Curvularia geniculata</i>	20.0	33.3	20.0	0.0	66.7
<i>Colletotrichum gloeosporioides</i>	13.3	33.3	26.7	60.0	46.7
<i>Fusarium equiseti.</i>	66.7	60.0	80.0	66.7	53.3
<i>Lasiodiplodia theobromae</i>	26.7	13.3	20.0	33.3	13.3
<i>Rhizopus oryzae</i>	80.0	53.3	80.0	33.3	33.3
<i>Trichoderma sp.</i>	0.0	-	6.7	-	-
Total	10	10	11	9	10

Mean percentage occurrence of fungal isolates in the study area

In general, *C. lunata* was the most predominant with the highest percentage occurrence (69.3%) in the study area (Fig. 2). It was followed by *F. equiseti* and *A. flavus* (65.3% each). The fourth most frequently occurring fungus was *A. oryzae*, with a mean percentage occurrence of

55.0% followed by *C. gloeosporioides* (36%), *A. fumigatus* (31.9%), *A. alternata* (31.8%) and *C. geniculata* (28%). *Lasiodiplodia theobromae*, *A. niger* and *Trichoderma* sp., were the three least occurring fungi with mean percentage occurrence of 21.3%, 19.9% and 1.3% respectively (Fig. 2).

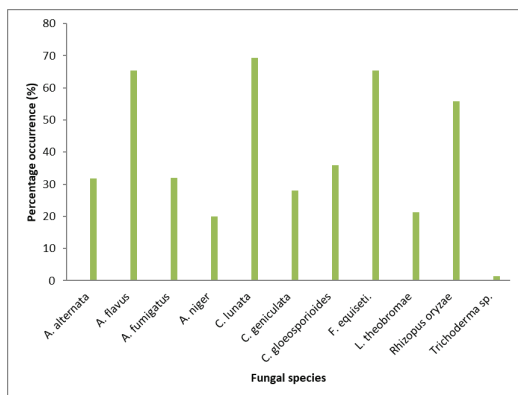


Fig. 2. Mean percentage occurrence of fungal species on farmer saved rice seeds in the irrigated rice production area of the Coastal Savannah of Ghana

Percentage infection of farmer saved seeds

A total of 75 samples of farmer saved seeds were collected from the study area. All the samples were contaminated with at least one type of fungal species, giving a percentage infection of 100% in all the five locations (Table 4).

TABLE 4

Percentage infection of farmer-saved rice seeds collected from different locations in the Coastal savannah zone of Ghana

Location	Number Of Seed Samples Collected from Farmers	*Number Of Samples Infected	Percentage Infection (%)
Afife	15	15	100.0
Akuse	15	15	100.0
Ashiaman	15	15	100.0
Asutuare	15	15	100.0
Kpong	15	15	100.0

*Infected sample is the one in which at least one type of fungi was detected on the seeds after blotter tests.

Discussion

Several fungal pathogens have been recorded worldwide on farmer-saved rice (Mew and Gonzales, 2002; Kumar *et al.*, 2014) and

their incidence on these seeds has been demonstrated to correlate negatively with percentage germination and seedlings vigour (Haque *et al.*, 2007; Kumar *et al.*, 2014). In this study, 11 seed borne fungal species from 8 different genera were recorded from a total of 75 samples of farmer saved seeds obtained from the coastal savannah zone of Ghana. Osumanu, (2012) identified 12 different fungi on farmer saved seeds from some selected farmers in the same study area. In India, as many as 30 different fungi were identified from farmer saved seeds of paddy rice (Kumar *et al.*, 2014). Results from this current study is a further confirmation that farmer-saved seeds are often contaminated with fungal species.

Results from this study showed infection of farmer-saved seeds by fungi was very high. Every seed sample collected from the study area harbored at least one fungal (Table 4). This high incidence of fungal species on the farmer-saved seeds could be an indication that the farmers were not storing their seeds under good hygienic conditions (Kumar *et al.*, 2014). In such a case, there is a high likelihood that these seeds will perform poorly in terms of percentage germination and seedlings vigour. Eventually, this will impact negatively on plant populations in the field and overall yield from fields planted with such seed. Indeed, reports from farmers in these areas indicate lower average yields per unit of land compared to other irrigated rice production areas of the world. There is therefore, the need for farmers in these areas to be trained on how to produce and store their seeds. Information available have shown that, when farmers are trained on good seed production and storage methods, their seeds are healthier and show higher percentage germination and seedlings vigour (Haque *et al.*, 2007).

All the 11 fungal species obtained in this study have been associated with either seeds of rice or of other crops such as legumes and other cereals (Nutsugah *et al.*, 2004; Haque *et al.*, 2007; Kumar *et al.*, 2014). Generally, *C. lunata* was one of the most prevalent fungal species found in the rice seeds in the study area. The pathogen is an important seed borne fungus in rice worldwide (Kim and Lee, 1998; Parimelazhagan & Francis, 1999). In Ghana, it has been reported in all the major agro-ecological zones where rice is cultivated, including the Coastal Savannah Zone (Osuman, 2012). This fungus and its close relative, *Curvularia protuberata* has been reported as a seed-borne pathogen which cause germination failure and spotting of rice grain (Sisterna & Dal Bello, 1998). The identification of *C. lunata* in this study underscores the fact that the fungus is gradually becoming an important pathogen in rice production in Ghana. Apart from *C. lunata*, other fungal species, known to cause diseases in rice fields and which were isolated in this study were *C. geniculata* (causing diseases on the inflorescence and grains of rice) and *Alternaria alternata* causing foliage diseases (Mew and Gonzales, 2002). These pathogens could therefore be contributing to the high incidence of diseases of rice plants currently being reported by farmers in the irrigated rice ecology of the coastal Savannah zone of Ghana (Honger: unpublished data).

Other fungal species found on the farmer saved rice seeds in the study area including *F. equiseti*, *R. oryzae*, *A. flavus*, *A. fumigatus*, *R. oryzae* and *Trichoderma sp.* were reported on rice seeds in previous works (Osuman, 2012; Signaboubo, 2016). However, their pathogenicity potential has not been demonstrated in the field (Mew & Gonzales, 2002). In spite of this, these contaminant fungi

need to be studied in the future as changes in environment and in the genetic make-up of an organism, which was once harmless, could result in damages to the host (Gupta *et al.*, 2017). Two fungal species, *L. theobromae* and *C. gloeosporioides* isolated in this study are not commonly found associated with rice seeds but have been reported to cause rot of plant tissues including the stem, leaves and floral parts of crops such as sugarcane and onions (Agrios, 2005; Honger *et al.*, 2014). Their presence in rice seeds in the study area could be potentially problematic as they could cause massive damage to rice plants in the field and therefore deserve attention.

With the exception of *Trichoderma sp* which was found only on seeds from Ashiaman, there were almost the same diversity of seed-borne fungal species across the five locations, varying only in the percentage occurrences (Table 2). Generally, the types of fungal species present on the seeds at a location may be influenced by the prevailing environmental condition, particularly, the humidity at the time of harvesting (Naqvi *et al.*, 2013). However, since all the five locations selected in the study were found in the same agro-ecological zone, fungal diversity could have been influenced by the same environmental conditions. In addition, there is evidence of sharing of seeds among the farmers, and cultural practices in the area were the same among farmers. Presumably, these could have played a role for the similarity of fungi on rice seeds found in the five areas under study

Previous studies by Osumanu, (2012) identified almost the same types of fungal species on rice seeds in the country. Her identification was based mainly on cultural and morphological features of the fungi. In this study, molecular method was used to augment the cultural and morphological

identification to remove all ambiguities. In this study, distinguishing between *C. lunata* and *C. geniculata*, and among the *Aspergillus* species, solely on the cultural and morphological features would have been very difficult. This is due to the fact that members of these two groups of fungi possess cultural and morphological features, which apart from being influenced by environmental factors also overlap among genetically distinct species (Balajee *et al.*, 2005). However, the application of the sequence analysis of the ITS region, made it possible for all isolates to be identified to the species level. The ITS region has been and remains an important barcode for the delineation of the species status of unknown isolates (Damm *et al.*, 2000) due to its ability to clearly define interspecific variations among even closely related species (Lin *et al.*, 2011). In disease diagnosis and control, proper and accurate identification of causative organisms is crucial (Martin *et al.*, 2000) and therefore, application of the sequence analysis of the ITS region in this study to identify the fungal species infecting rice seeds gives further credence to their identities.

Conclusion

Eleven (11) different fungal species belonging to eight (8) genera were identified as contaminants of farmer-stored seeds using a combination of conventional and molecular sequencing of the ITS region of the species isolated from the farmer-saved rice seeds in the study area. The predominant fungal species varied from one distinct sampling site to another although *C. lunata* was generally found in all the sampling sites (33.0-93.3%) while *Trichoderma* sp was only recorded in seeds from Ashaiman (6.7%). It is evident that the seed health quality of the farmer-saved seeds from the Coastal Savanna Zone

of Ghana leaves much to be desired and would invariably influence negatively on the germination capacity, seedling establishments and yield of the crop. We recommend a regular training in modern seed production and storage technologies for the farmers to improve their crop yield. The supply of certified quality seeds to the farmers will also ameliorate possible low yield challenges facing the farmers in the area.

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