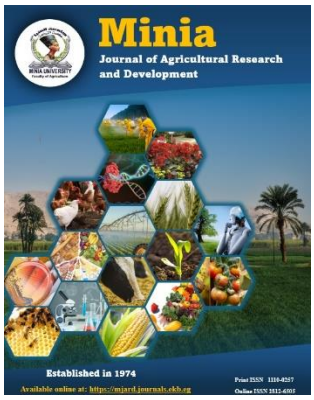
Minia J. of Agric. Res. & Develop., Minia Univ., Vol. 44 (4): 705 - 720, 2024

Minia Journal of Agricultural Research and Development

Journal homepage & Available online at:
https://mjard.journals.ekb.eg



Characterization and genetic diversity of *Curvularia lunata* isolates associated with sorghum grains mold in Egypt

Abbas, Abeer. H. and Agag, Sayed. H.
Maize and Sugar Crops Diseases Research Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

Received: 20 Dec. 2024 **Accepted: 2 Jan. 2025**

ABSTRACT

Sorghum bicolor, an important crop in arid and semi-arid areas, faces significant biotic stressors, particularly grain mold caused by fungal infections. *Curvularia lunata* emerged as a prominent pathogen in sorghum leaf spot and grain mold, significantly impacting grain quality and yield. This study aimed to isolate and characterize *C. lunata* strains from sorghum grain obtained from Egyptian markets. Ten isolates were found, and tests on sorghum cultivar Giza 15 showed a wide range of virulence, with disease ratings ranging from 22.67% to 66.68%. Morphological identification and phylogenetic investigation verified the isolates' identity as *C. lunata*, with RAPD-analysis revealing the genetic differences between isolates. Phylogenetic analysis divided the isolates into two major groups, with considerable genetic similarity within each group. However, some isolates revealed significant genetic divergence. The RAPD profiling revealed 38 DNA fragments, 93.05% of which were polymorphic, showing that the isolates studied had a high level of genetic diversity. This diversity demonstrates the pathogen's adaptability, which may be connected to pathogenic potential in various conditions. The findings provide critical insights for breeding resistant sorghum varieties and underscore the need for comprehensive management strategies to mitigate grain mold's impact on sorghum production.

Keywords: Sorghum grains mold, *Curvularia lunata*, Molecular identification, RAPD, Phylogenetic analysis

INTRODUCTION

Sorghum (*Sorghum bicolor* [L.] Moench) is the 5th most important world cereal crop after maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), and barley (*Hordeum vulgare* L.) (FAO 2019). This resilient crop can thrive across various latitudes and is a vital source of animal feed, traditional and processed foods, beverages, and biofuels. Sorghum is critical in the drier tropics, where it is the fifth most crucial cereal, providing essential calories for millions (Frederiksen and Odvody, 2000).

In Egypt, sorghum is grown in different ecological zones due to its adaptability to

*** Corresponding author: Abbas, Abeer. H.**
E-mail address: abeer_hamdy@ymail.com

various environmental conditions (salinity, high temperature, and drought stressors). The country's sorghum productivity is 0.88 tons/ ha, which is relatively low compared with the world average of 1.45 ton/ha and the USA's 4.58 ton/ha (FAO, 2019).

Despite its significance, sorghum production faces numerous challenges due to abiotic and biotic stresses. Grain mold, a fungal disease complex, poses a significant constraint to sorghum productivity and grain quality worldwide, particularly in humid and warm environments during the rainy season (Bandyopadhyay and Chandrashekar, 2000; Garud et al. 1998; Singh and Bandyopadhyay, 2000). Although, fungi in several genera have been associated with grain mold (Bandyopadhyay and Chandrashekar, 2000; Esele et al., 1995; Singh and Bandyopadhyay,2000), *Fusarium thapsinum* Klittich, Leslie, Nelson, and Marasas and *Curvularia lunata* (Wakk.) are considered the most common pathogenic agents (Singh and Bandyopadhyay, 2000) however, the prevalence and severity of diseases caused by these pathogens can differ significantly based on geographic location and climatic conditions (Bandyopadhyay and Chandrashekar, 2000; Forbes et al., 1992; Singh and Bandyopadhyay, 2000). Yield losses attributed to grain mold ranging from 30-100% may occur with severe epiphytotic (Singh and Bandyopadhyay, 2000). Fungi in genera *Colletotrichum, Alternaria*, and *Phoma* are frequently recovered from moldy grains, but their significance appears insignificant (Forbes et al 1992).

Curvularia. lunata, has been identified as a dominant pathogen on sorghum and finger millet seeds collected in Korea, potentially serving as a primary source of infection (Yago et al., 2011). It is responsible for not only grain mold in sorghum (Tarekegn et al., 2006; Sharma et al., 2010) but also leaf blight in other crops such as pearl barley (Dai et al., 2019) and leaf spot in cotton (Shirsath et al., 2018).

Furthermore, mold fungi that develop on seeds produce mycotoxins, posing health risks to humans and animals (Halt, 1994). Fungi-induced discoloration of sorghum seeds results in poor quality, diminishing market acceptability, and economic value (Castor and Frederiksen, 1980; Gopinath and Shetty, 1987). Grain mold decreases seed size and weight and negatively impacts grains' nutritional quality and storage viability (Gopinah, 1984; Bandyopadhyay, 1986).

Management strategies, including avoidance, chemical seed treatments, and resistant cultivars, have proven effective in mitigating the impact of grain mold disease complex (Forbes et al., 1992). Among these, employing resistant cultivars is the most effective means of controlling grain mold. However, the mechanisms of grain mold resistance in sorghum are complex and multifaceted (Castor, 1981; Forbes et al., 1992; Waniska et al., 2001). Several kernel characteristics can enhance resistance, including grain hardness, pigmented testa, red pericarp, endosperm texture, high tannin content, and elevated levels of flavan-4-ol (Mukuru, 1992; Waniska et al., 2001). Additionally, studies have indicated that antifungal proteins such as sormatin, chitinases, glucanases, and ribosome-inhibiting proteins contribute to the mechanisms of resistance against grain mold (Bueso et al., 2000; Rodriguez-Herrera et al., 1999; Seetharaman et al., 1996). The risk of introducing new species or strains of a pathogen (s) can be significantly reduced with a thorough understanding of their seed-borne characteristics and appropriate treatments to minimize seed-transmitted inoculum. This study aimed to isolate and identify the main fungal pathogen (s)

causing grain mold in sorghum grains from the Egyptian retail market. The pathogen (*C. lunata*) was isolated from infected grains and tested for pathogenicity. The most virulent strains were identified using PCR with ITS primers and sequencing. Additionally, the study evaluated the susceptibility of various sorghum varieties and the pathogen's host range, focusing on the *Poaceae* family.

MATERIALS AND METHODS

Sample Collection and Fungal Isolation

Sorghum grain (Giza 15 cultivar) samples were gathered from various locations across Giza Governorate, Egypt during June-September 2022. The samples were purchased from warehouses and local markets, with three replicates taken from three different spots at each location. Each sample (100 grains) was kept in a new paper bag, promptly transported to the laboratory, and stored at 4°C for fungal analysis.

Grains of each sample, individual, were disinfested with a 0.5 % sodium hypochlorite solution for 2 min, rinsed three times in sterilized distilled water (SDW)for 2 min. and then transferred to potato dextrose agar (PDA) amended with 125 ppm of Ceftriaxone antibiotic (Sandoz, Egypt). The plates were incubated at 26 ± 1 ºC for 4 days. Colonies originating from surface-sterilized grains were isolated via hyphal tip transfer for subsequent analysis (Sallam and Abo-Elyousr, 2012).

Morphological characterization

All purified isolates were identified based on their macro- and micro-morphological characteristics according to the keys of Sivanesan (1987), Ellis (1971), Barnett and Hunter (1972) and Manamgoda *et al*. (2012).

Fungal frequency

Isolation was carried out from 1200 sorghum grains taken randomly from Giza 15 cultivar, as previously mentioned. The frequency of this associated fungi was estimated.

Pathogenicity test

A pathogenicity test of isolates was carried out on the foliage of 4-week-sorghum seedlings (cv. Giza 15) under greenhouse conditions at the Maize and Sugar Crops Diseases Research Department, Plant Pathology Institute, Agricultural Research Center. A spore suspension (1 x 10^6 conidia/ml) of each of the selected isolates was prepared by blending the mycelium of the obtained isolates, individually, and filtered through cheesecloth. The sorghum seedlings were inoculated by spraying a spore suspension on the leaves. Control plants were treated with SDW. The 10 plants were then inoculated per isolate. All inoculated seedlings were covered by clear plastic bags and maintained in the greenhouse overnight for 48 h at 28 ± 2°C, and were observed daily until disease symptoms were visible (Kumar et al., 2017). The disease severity index was evaluated at 30 days post-inoculation as follows:

The disease index was calculated by adopting disease-rating scale using a 0 - 10 scale with 0 = leaves without any spot (controls), 1 = spots occupying 1-10 %, 2 = 11-20%, 3 = 21-30%, and 4 =31-40% %, 5 = 41-50%, 6 =51-60%, and 7 =61-70%, 8 = 71-80%, 9 =81-90%, and 10 =91-100% of the leaf area.

The mean of disease severity index (DSI) for each replicate was calculated by the formula suggested by Lue *et al*. (1995) as follows:

Disease severity (%) = Σ (n x r) / NR x 100

Where n = number of infected leaves on the plant, r = numerical rate of infected leaves, N total number of leaves on the plant, and R maximum numeric rate **(Lue *et al*. (1995).**

Koch's postulates were fulfilled as the fungus was re-isolated from the leaf tissues artificially inoculated with pieces of necrotic leaves.

Molecular characterization of *Curvularia* isolates

Fungal growth

Three small pieces of PDA agar containing mycelia and spores were placed into a 250-ml conical flask with 50 ml of potato dextrose broth (PDB). The liquid cultures were incubated at 25°C for 4 days with constant shaking at 100 rpm. Mycelia were collected by filtering the fungal culture through the Whatman No.2 filter paper. Excess water was removed by pressing the filter paper with mycelia between layers of tissue paper. The semi-dried mycelia were then scraped off the filter paper and stored at -40°C until DNA extraction.

DNA Extraction

Mycelia were ground using a mortar and pestle under liquid nitrogen, and the result was transferred into 1.5 ml Eppendorf tubes, ensuring each tube was no more than one-third full for optimal results. Using the CTAB method, genomic DNA was extracted (Murray and Thompson, 1980). The DNA's quality and quantity were verified on 1% agarose gel stained with 1 µg/ml acridine orange (Sambrook et al., 1989). The DNA was diluted to a final concentration of 20 ng/µl.

PCR and phylogenetic studies

The ITS-rDNA was amplified and sequenced using the following primers: ITS4 and ITS5 (White et al., 1990). The PCR reaction mix included 1 ng/µl genomic DNA, 1× PCR buffer (Bioline US Inc., USA), 1.5 mM MgCl2, 0.2 mM dNTPs, 0.25 µM of each primer, and 1.0-unit Taq DNA polymerase (Bioline), in a final volume of 30 µl. The PCR program was as follows: an initial cycle at 94°C for 3 minutes, followed by 30 cycles at 94°C for 1 minute, with a final extension at 72°C for 5 minutes. PCR was conducted using a TC-412 thermocycler (Techne, UK). The PCR products were separated on 1.5% agarose gel in 0.5× TBE buffer stained with 1 µg/ml acridine orange, visualized under UV, and photographed. Products were purified and sequenced at the sequencing service unit of Macrogen Inc. (Macrogen Inc., Seoul, Korea). DNA sequences were edited and aligned using BioEdit software, with single nucleotide polymorphisms (SNPs) identified within and between fungal DNA sequences. Phylogenetic analysis was performed using PAUP software (Swofford, 2002), where neighbor-joining (NJ) and parsimonious trees (using heuristic search) were constructed for both individual and combined sequences. Bootstrapping with 1000 replicates was used to support tree branches (Felsenstein, 1985), and consistency and retention indices (CI and RI) were calculated for parsimonious trees in PAUP.

RAPD Analysis

RAPD reactions were conducted following the protocol by Williams et al. (1990). Each PCR reaction was prepared in a final volume of 15 µL, containing: 2.5 µl master-mix (Soles biodyne), 2 µl for each primer of 10 µm, 0.5 µl DNA of 100 ng, and 10 µl sterilized double distilled water. The sequences of the RAPD primers used are listed in Table 1. RAPD reactions were carried out in an Applied Biosystems 2720 thermocycler. The RAPD-PCR cycling conditions were initial denaturation at 94°C for 5 minutes, followed by 40 cycles of

94°C for 1 minute, 36°C for 1 minute, and 72°C for 2 minutes, with a final extension at 72°C for 10 minutes. PCR products were then analyzed via electrophoresis on 1.5% agarose gels stained with 0.5 µg/mL ethidium bromide.

Table 1. List of RAPD primer sequences used in this study.

Primer code	(G+C) 100 %	Sequence
1	70	CACGGCGAGT
2	60	GTCGATGTCG
3	70	AAGCCTCCCC
4	70	CGTCGCCCAT
5	60	GGGTTTGGCA
6	60	AGCGAGCAAG

Varietal response

Twelve sorghum cultivars (Giza 15, 305 T.w.c, 306 T.w.c, Dorado, Shandaweel 1, Ahmos, Ramsees, Ezees, Shahd, Mabrook, Hourus, and Makka) were evaluated against *C. lunata*. Grains were taken from Crop Production Research Institute, ARC, Giza. Grains were cleaned with 70% ethanol for 30 s, then in 1% bleach for one minute, rinsed with distilled water, and then sown in 30 cm plastic pots, with ten seeds of each cultivar sown per pot and later thinned to four healthy seedlings. *C. lunata* inoculum was prepared at a concentration of 10^6 spores/ml. At 30 days post-emergence, plants were sprayed with the inoculum and covered with plastic bags. Four weeks post-inoculation, plants were assessed for disease development. The disease index was calculated by adopting the disease-rating scale as aforementioned described.

The mean of disease severity index (DSI) for each replicate plant was calculated by the formula suggested by Lue *et al*. (1995) as mentioned before.

Host Range:

This study used five species of plants: sorghum (Giza 15), maize (bushy), Soudan grass (single hybrid 107), sugar corn (super sweet single hybrid 110) and rice (S. 101). Grains of the tested plants were kindly obtained from Crop Production Research Institute, ARC, Egypt. The teste was carried out in pot No. 30 containing steam-sterilized soil. Seedlings were artificially inoculated by isolating no. C_1 as mentioned before. Three replicates per treatment were used. Plants were observed, and disease symptoms were recorded 30 days post-inoculation. Disease severity and disease rating were calculated as mentioned before.

Statistical analysis

The statistical analysis for ANOVA was done using the Minitab program version 17 (Minitab. 2010), while the cluster analysis was done using the SPSS program version 17 (SPSS 25.0 2021).

RESULTS

Isolation and frequency of isolated fungi

Data in Table (2) shown that four hundred and ninety fungal isolates were isolated and purified from samples of sorghum grains which collected from El Giza markets. *Curvularia spp*. is the dominant pathogen in diseased sorghum-grain (37.76%), followed by *Aspergillus*

spp. (29.59%), *Fusarium spp*. (18.37%), *Alternaria spp*. (8.16%) and *Helminthosporium spp*. as the least one represented 4.08%. Also, non-identified fungi represented about 2.04% of the total frequency of isolated fungi**.**

Table 2. Frequency of different fungi associated with grain mold of sorghum.

Fungi	Number of isolates	Frequency (%)
Curvularia spp.	185	37.76
Aspergillus spp.	145	29.59
Fusarium spp.	90	18,37
Alternaria spp.	40	8.16
Helminthosporium spp.	20	4.08
Other fungi	10	2.04
Total	490	100

Pathogenicity test of *Curvularia spp*. isolates

Due to the fungus *Curvularia spp*. which was the dominant fungal isolated from grains of sorghum, the ten isolates of *Curvularia spp*. designated C_1 through C_{10} were successfully obtained and assessed for their pathogenicity on sorghum cv. Giza 15. Pathogenicity testing revealed variation in disease severity across the isolates, as measured by the disease index (Table 3 and figure 1). Isolate C_1 exhibited the highest virulence, with a disease index of 66.68%, making it the most aggressive among the isolates. This was followed by C_2 and C_3 isolates, both with a disease index of 52.00%, indicating similarly high pathogenic potential.

Moderate levels of pathogenicity were observed in isolates C_4 and C_5, with disease indices of 43.00% and 40.33%, respectively. The remaining isolates (C_6 to C_{10}) displayed lower pathogenicity, with disease indices ranging from 22.67% to 30.67%. Notably, isolate C_9 was the least virulent, with a disease index of 22.67%, suggesting a reduced pathogenic impact on the host. These results indicate substantial variability in pathogenicity among the *C. lunata* isolates, with C_1, C_2, and C_3 being the most virulent on sorghum cv. Giza 15. This information is critical for understanding the potential severity of *C. lunata* infections and for guiding breeding programs aimed at developing resistant sorghum cultivars.

Table 3. Pathogenicity test of *Curvularia lunata* isolates to sorghum cv. Giza 15.

Fungal isolates	Disease index
C. lunata-C_1	66.68^{a}
C. lunata-C_2	52.00^{ab}
C. lunata-C_3	52.00^{ab}
C. lunata-C_4	43.00^{bc}
C. lunata-C_5	40.33^{bcd}
C. lunata-C_6	30.33^{cd}
C. lunata-C_7	29.33^{cd}
C. lunata-C_8	30.67^{cd}
C. lunata-C_9	22.67^{d}
C. lunata-C_{10}	27.00^{cd}

Figure 1. Pathogenicity test of *C. lunata* isolate C_1) on sorghum leaves, the growth on PDA (left) and artificial infection on Giza 15 cv. (right).

Morphological and Phylogenetic Identification

Morphological identification, based on Sivanesan (1987), Ellis (1971), Barnett and Hunter (1972) and Manamgoda *et al*. (2012) and revealed characteristics typical of the *Curvularia* genus. Colonies on PDA medium were observed to have a cottony to thin mycelial appearance, with colors ranging from black and moss green to gray (Figure 1, left). Microscopically, conidiophores were septate, varying from straight to bent, and conidia were observed after 10 days of incubation at 25 °C ± 2 °C. Figure 2 illustrates these morphological characteristics, including colony morphology and conidial structures. The sequences were deposited in Gen Bank under accession numbers PP141338, PP141339, PP141340, PP141341, PP141342, PP141343, PP141344, PP141345, PP141346 and PP141347 for the obtained isolates C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9 and C_{10}, respectively. The phylogenetic analysis of our fungal isolates revealed distinct clustering patterns, indicating their genetic relationships. The tree was generated using the neighbor-joining algorithm, and the bootstrap values were calculated from 1000 replicates (Fig. 3). Most of the isolates were grouped within the *Curvularia* genus. Our isolates were divided into two clusters supported by a 99% bootstrap value. The first one contained most of the isolates (Isolates 1-9), which were grouped as a separate branch with a strong level of confidence (99% bootstrap support). The isolates are determined to be closely related and classified as *Curvularia lunata* based on their sequence similarity with known strains of *C. lunata* (AUMC 15458 and isolate Iz-7). The second one, with isolate number 10 formed a distinct subgroup, however, it remained within the larger group of *C. lunata*, suggesting some genetic variation but overall resemblance to the *C. lunata* group. The reference strains, including *C. pseudorobusta*, *C. robusta, C. spicifera, C. australiensis, C. oryzae*, and *C. siddiquii*, were found to have distinct branches with strong bootstrap support (ranging from 88% to 98%). This suggests that they have clear genetic differences from the main clades of *Curvularia* isolates.

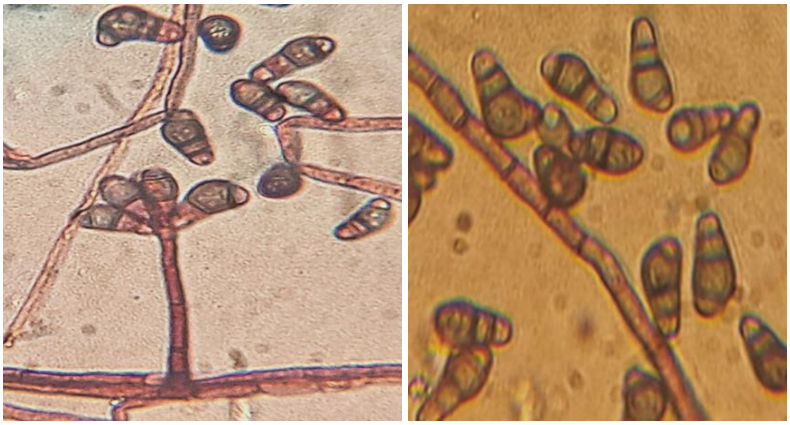


Figure 2. Conidial phase of *Curvularia lunata*

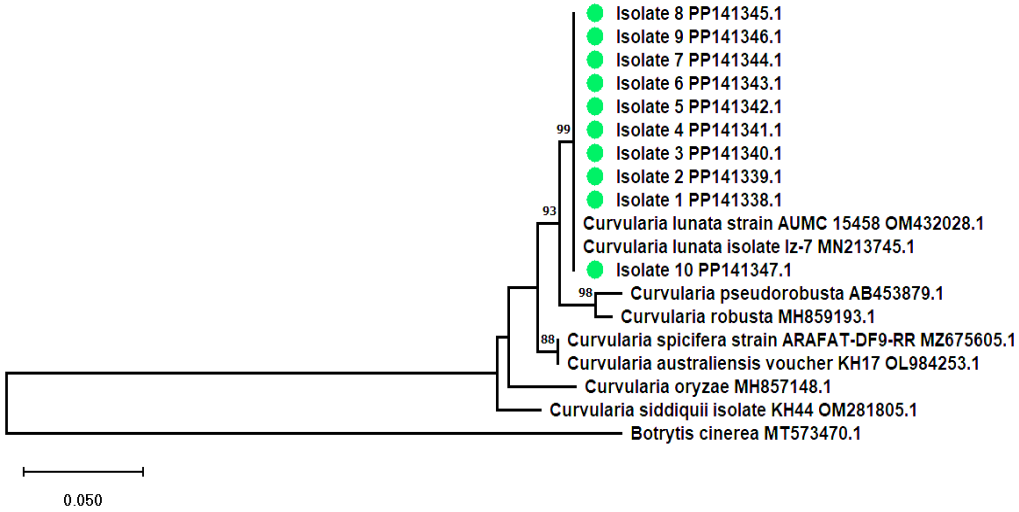


Figure 3. Phylogenetic tree of representative sequences of the amplified Ras*Clg2p* protein gene from *Curvularia lunata* DNA isolated from sorghum.

The genetic similarity among the ten *C. lunata* isolates was assessed using six RAPD primers, producing 38 DNA fragments with an average of 6.3 bands per primer. Of these fragments, 93.05% were polymorphic, indicating a high level of genetic diversity among the isolates. The polymorphic information content (PIC) values ranged from 0.32 to 0.36, with an average PIC of 0.34, suggesting the primers effectively captured variability among the isolates. The similarity matrix (Table 4) revealed genetic relationships between the isolates, with the highest similarity (100%) observed between isolates 4 and 5, while the lowest similarity (44%) was between isolates 8 and 10.

This variability highlights genetic distinctions among isolates within the *C. lunata* species. A dendrogram based on Dice's coefficient (Fig. 4) was generated to visualize the genetic relationships and clustering patterns among the isolates. The cluster analysis divided the ten isolates into six distinct groups: Cluster 1 contained isolates 4, 5, and 8, showing close genetic similarity, Cluster 2 consisted of isolate 1,

Cluster 3 included isolates 2, 3, and 6, indicating moderate similarity, Cluster 4 included isolate 7, Cluster 5 comprised isolate 9, and Cluster 6 contained isolate 10, representing the most genetically distinct isolate.

The efficiency of the RAPD primers varied, with primers 1, 3, 5, and 6 showing 100% polymorphism, indicating their effectiveness in differentiating the genetic variability among isolates. Primer 2 exhibited 83.33% polymorphism, while primer 4 showed 75% polymorphism, still contributing valuable genetic data. Overall, the results demonstrate significant genetic diversity among the *C. lunata* isolates, which may correlate with pathogenic variability and adaptability across different environments. The clustering patterns suggest possible subgroupings within *C. lunata*, which could be further explored for insights into functional adaptations and evolutionary relationships.

Table 4. The similarity matrix for ten isolates of *Curvularia lunata* based on RAPD data.

Case	Isolate1	Isolate2	Isolate3	Isolate4	Isolate5	Isolate6	Isolate7	Isolate8	Isolate9	Isolate10
Isolate1	1.000									
Isolate2	.595	1.000								
Isolate3	.667	.895	1.000							
Isolate4	.667	.643	.690	1.000						
Isolate5	.667	.643	.690	1.000	1.000					
Isolate6	.632	.865	.821	.667	.667	1.000				
Isolate7	.581	.667	.750	.480	.480	.710	1.000			
Isolate8	.619	.537	.651	.754	.754	.524	.514	1.000		
Isolate9	.529	.606	.629	.566	.566	.529	.593	.632	1.000	
Isolate 10	.486	.667	.579	.643	.643	.595	.533	.439	.485	1.000

Table 5. Total number of bands (TNB), polymorphic bands (PB), percentage of polymorphic bands (PPB)and polymorphic information content (PIC) for the six RAPD primers.

RAPD Primer	TNB	PB	PPB	PIC
1	10	10	100	0.36
2	6	5	83.33	0.32
3	5	5	100	0.35
4	4	3	75	0.32
5	6	6	100	0.36
6	7	7	100	0.36
Total	38	36	----	----
Average	6.3	6	93.055	0.34

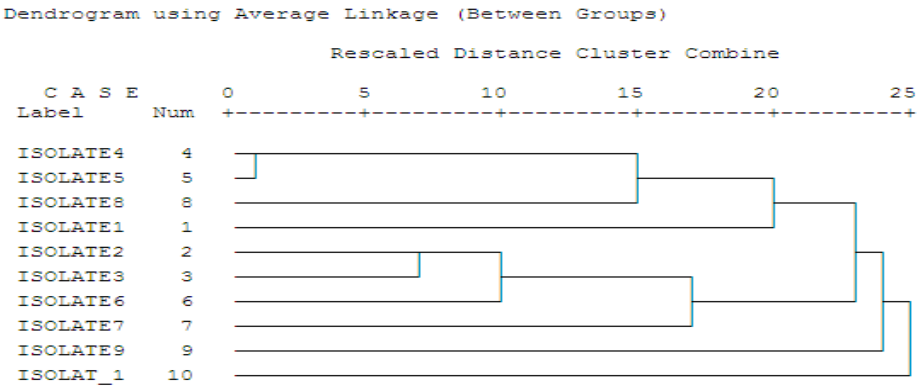


Figure 4. Dendrogram of cluster analysis based on RAPD data using Dice coefficient for the ten isolates of *Curvularia lunata*.

Varietal response of sorghum cultivars to *Curvularia lunata* infection

The pathogenicity of *Curvularia lunata* (isolate C_1) on different sorghum cultivars revealed varied responses to infection, as shown in Table 6. Among the tested cultivars, Giza 15 cultivar exhibited the highest susceptibility with a disease index of 53.33%, indicating it is highly vulnerable to *C. lunata*. Other cultivars, such as T.W.C 305 cultivar and T.W.C 306 cultivar, also showed moderate susceptibility, with disease indices of 41.00% and 42.33%, respectively. In contrast, Dorado cultivar demonstrated the highest resistance among the cultivars, with a disease index of only 7.33%, making it the least affected by the pathogen. Other cultivars, including Ahmos, Shandaweel 1, Eseas, Shahd, and Mabrook, exhibited intermediate resistance, with disease indices ranging from 14.33% to 29.00%. Cultivars like Ramsees, Horus, and Makka showed moderately higher susceptibility, with disease indices between 30.33% and 34.33%. These results indicate significant variation in susceptibility among the sorghum cultivars, with Giza 15 being the most susceptible and Dorado the most resistant. This information is valuable for selecting resistant cultivars in breeding programs aimed to control leaf spot and grains mold diseases caused by *C. lunata*.

Table 6. Reaction of some sorghum cultivars against *Curvularia lunata* isolate C_1.

Sorghum cultivars	Disease index
Giza 15	53.33^{a}
T.W.C 305	41.00^{ab}
T.W.C 306	42.33^{ab}
Shandaweel 1	24.67^{bcd}
Dorado	7.33^{b}
Ahmos	14.33^{cd}
Ramsees	30.33^{a-d}
Ezeas	23.00^{bcd}
Shahd	24.67^{bcd}
Mabrook	29.00^{a-d}
Horus	30.67^{a-d}
Makka	34.33^{abc}

Host Range of *Curvularia lunata* isolate C_1.

The pathogenicity test conducted on different host plants to assess their susceptibility to *C. lunata* (isolate C_1) showed varying degrees of infection, as indicated by the disease index values in Table 7. Rice (S. 101) was the most susceptible host, with the highest disease index of 44.33%, closely followed by sorghum (Giza 15) with a disease index of 43.67%. Sugar corn (S. H 110) also showed a moderate level of susceptibility, with a disease index of 37.67%. Soudan grass (S.H 107) exhibited a lower susceptibility, with a disease index of 27.33%, while maize (Boushy) had the lowest disease index of 25.00%, indicating relatively better resistance among the tested hosts.

These results suggest that *C. lunata* (isolate C_1) has a broad host range, with varying levels of infection across different plant species, highlighting the potential risk of cross-infection in agricultural settings where these crops are grown.

Table 7. Host Range of *Curvularia lunata* (Isolate C_1).

Hosts	Disease index
Maize (Boushy)	25.00^{c}
Sorghum (Giza 15)	43.67^{a}
Soudan Grass (S.H 107)	27.33^{c}
Sugar corn (S. H 110)	37.67^{b}
Rice (S. 101)	44.33^{a}

DISCUSSION

This study provides significant insights into the complex relationships of genetic diversity, pathogenic variability, and host vulnerability within the *C. lunata*-sorghum pathosystem. The significant genetic variety among the ten isolates, as proven by RAPD analysis, is an important finding. The high average PIC values of 0.34 indicated an effective capturing of genetic diversity by the RAPD markers. This diversity demonstrates the evolutionary plasticity of the pathogen with potential impacts on its virulence and host range across different climates and agronomic conditions. The existence of two primary phylogenetic clusters and a genetically distinct subgroup denoted by isolate C_{10} supports the hypothesis that *C. lunata* populations might exhibit restricted adaptations.

The findings correlate with the investigations conducted by Manamgoda et al. (2012) and Yago et al. (2011), which similarly emphasized the genetic variability of *Curvularia spp*. as a factor leading to their pathogenic efficacy.

The data of pathogenicity trials demonstrated significant variation in disease severity, with isolates C_1 and C_2, whereas isolate C_9 was low pathogenic. This variability emphasizes the importance of identifying pathogen populations before initiating control measures. The correlation between genetic diversity and pathogenic capability, as demonstrated in this study, has been documented for other fungal diseases such as *Fusarium spp.* (Bandyopadhyay et al., 2000; Forbes et al., 1992).

Knowing these relationships can facilitate the prediction of disease outbreaks and guide breeding strategies to emphasize resistance to the most virulent pathogen genotypes.

The assessment of sorghum cultivars against *C. lunata* showed significant diversity in resistance levels. Dorado cultivar appeared as a promising cultivar with the lowest disease score of 7.33%, whereas Giza 15 cultivar exhibited significant susceptibility at 53.33%. The variation in resistance levels among cultivars is due to changes in kernel characteristics, including hardness, pericarp color, and tannin concentration, which are linked to defense against grain molds (Mukuru, 1992; Waniska et al., 2001). Identifying cultivars with high resistance, such as Dorado, is essential for breeding initiatives to provide long-term *C. lunata* resistance.

The host range study indicated that *C. lunata* isolate C_1 may infect several crop species, with notable disease indices recorded for rice (44.33%) and sorghum (43.67%). This extensive host range corresponds with previous findings of *C. lunata* infecting several crops, such as grains and legumes (Sharma et al., 2010; Tarekegn et al., 2006). The variety of host infections poses challenges for integrated disease control, especially in areas using intercropping practices. These findings emphasize the necessity of crop rotation and host resistance as synergistic approaches to mitigate disease dissemination and longevity in the soil.

Our findings imply that the pathogen, which is characterized by genetic variety, can adapt while simultaneously having a high virulence potential. The findings emphasize the need for integrated pest control measures such as resistant cultivars, seed treatments, and crop rotation.

These differences in pathogen-host interactions underscore the need for a more in-depth investigation of sorghum's molecular and physiological mechanisms underlying resistance. High-throughput genomic methods, such as genome-wide association studies and transcriptomics, may aid in the understanding of underlying resistance mechanisms and identify candidate genes for resistance breeding.

Future research should focus on the environmental and biological causes of the *C. lunata* population structure at broader regional scales. There is still much to learn about how climatic parameters like temperature, humidity, and precipitation patterns influence pathogen virulence and host vulnerability. Using molecular approaches to characterize strain virulence diversity and better understand the environmental and genotypic elements influencing pathogen fitness will be critical for creating tailored management measures. The genetic and pathogenic variety of *C. lunata* populations in Egypt poses a significant challenge to sorghum-growing crops. Developing resistant cultivars and knowledge of pathogen biology and host-pathogen interactions can lead to effective disease control. *C. lunata* was identified as the fungus with the highest occurrence from sorghum grains taken from multiple species using morphological and molecular marker analysis. It is pathogenic to these sorghum species, causing foliar necrotic regions and grain mold. This information is critical for understanding the potential severity of *C. lunata* infections and for guiding breeding programs aimed at developing resistant sorghum cultivars. Our research provides critical information for breeding efforts to improve sorghum resilience and protect global food security.

REFERENCES

Bandyopadhyay R. 1986. Grain mold. In: Fredariksen, RA (ed) Compendium of sorghum diseases., Am. Phytopathol. Soc., St. Paul Minnesota, USA, pp. 36-38.

Bandyopadhyay R, Butler DR, Chandrashekar A, Reddy RK and Navi SS. 2000. Biology epidemiology and management of sorghum grain mold. In: Chandrashekar A, Bandyopadhyay R, Hall AJ (Eds) Proceedings of Consultative Group Meeting on Technical and Institutional Options for Sorghum Grain Mold Management, International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India, pp 34-71.

Bandyopadhyay, R., Chandrashekar, A. 2000. Biology and management of sorghum grain mold. In: Chandrashekar, A., Bandyopadhyay R., Hall A. J. (eds), Proceedings of Consultative Group Meeting on Technical and Institutional Options for Sorghum Grain Mold Management. 18-19 May. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India.

Barnett H. L. and Hunter B.B. 1972. Illustrated Genera of Imperfect Fungi,1-241 pp. Burgess Publishing Company, Minneapolis, Minnesota

Bueso, F.J., Waniska, R.D., Rooney, W.L., Bejosano, F.P. 2000. Activity of antifungal proteins against mold in sorghum caryopsis in the field. J. Agric. Food Chem. 48, 810-816.

Castor L.L., Frederiksen R.A. 1980. *Fusarium* head blight, occurrence and effects on sorghum yields and grain characteristics in Texas. Plant Dis., 64: 1017-1019.

Castor, L.L. 1981. Grain mold histopathology, damage assessment and resistance screening within Sorghum bicolor (L.) Moench lines. Ph.D. thesis, Texas A&M University, College Station, TX, USA. 192 pp.

Dai, Yu-Li, Lin Gan, Fu-Ru Chen &Xiu-Juan Yang. 2019. Leaf blight caused by *Curvularia coicis* on Chinese pearl barley (Coixchinensis) in Fujian Province, China, Canadian Journal of Plant Pathology, 41(1): 1-7.

Ellis MB. 1971. Dematiaceous Hyphomycetes. Kew, UK: Commonwealth Mycological Institute.

Esele JP, Frederiksen RA, Miller FR. 1995. Importance of plant colour and modifier genes in grain mould resistance in sorghum. J. E. Afr. Agric. For. 61:31-37.

FAO. 2019. FAO Statistical Database (online). Food and Agricultural Organization of the United Nations. Rome. http://www.fao.org/faostat/en/#data/QC (open in a new window) accessed 10 June2021.

Felsenstein, J. 1985a. Phylogenies and the comparative method. American Naturalist 125:1-15.

Felsenstein, J. 1985b. Confidence limits on phylogenies: an approach usingthe bootstrap. Evolution 39:783–79

Forbes, G.A., R. Bandyopadhyay and G. Garcia 1992. A review of sorghum grain mold. In: Sorghum and Millets Diseases: a Second World Review. pp: 253– 264. de Milliano, W.A.J., R.A. Frederiksen and G.D. Bengston, (eds.). International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502 324, Andhra Pradesh, India.

Frederiksen RA, Odvody GN. 2000. Compendium of Sorghum Diseases. The American Phytopathological Society, St. Paul, MN, USA.

Garud TB, Shinde BM, Syed Ismail and BN Aglave. 1998. Effect of delayed harvesting on grain mold development. International Sorghum and Millets Newsletter, 39: 106-107.

Gopinah, A. 1984. Seed-borne *Fusarium* diseases of sorghum. Ph.D. Thesis, University of Mysore, India, p. 263.

Gopinath A, Shetty H. S. 1987. Comparison of field and Laboratory evaluation of head mould of sorghum with special reference to *Fusarium*. Indian Phytopathol., 40: 52-55.

Halt M. 1994. *Aspergillus flavus* and aflatoxin B1 in flour production. Eur. J. Epidermiol., 10(5): 555-558.

Kumar V, Biswas SK, Chowdary VT, Kishan L, Naresh P. 2017. Induced synthesis of defense enzymes during induced resistance against early blight of potato using plant extracts as inducer. J Scien Res Rep 16:1-11. ISSN: 2320-0227.

Lue, L., Kloepper, J. W. and Tuzun, S. 1995. Induction of systemic resistance in cucumber against *Fusarium* wilt by plant growth promoting rhizobacteria. Phytopathol., 85:695-698.

Manamgoda D. S., Cai L., McKenzie E. H. C., Crous P. W., Madrid H., Chukeatirote E., Shivas R. G., Tan Y. P., Hyde K. D., 2012. A phylogenetic and taxonomic re-evaluation of the *Bipolaris* - *Cochliobolus* - *Curvularia* Complex. Fungal Diversity 56: 131-144.

Minitab. 2010. Minitab 17 statistical software. State college, PA: Minitab.

Mukuru, S.Z. 1992. Breeding for grain mold resistance. In: W.A.J. de Milliano, R.A. Frederkisen & G.D. Bengston (Eds), Sorghum and millets diseases: a second world review. pp. 273–285. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India.

Murray MG and Thompson WF. 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8: 4321–4325.

Rodriguez-Herrera, R., Waniska, R.D., Rooney, W.L. 1999. Antifungal proteins and grain mold resistance in sorghum with a non-pigmented testa. J. Agric. Food Chem. 47, 4802-4806.

Sallam NMA, Abo-Elyousr K AM. 2012. Blight disease of tomato plants under greenhouse and field conditions. Plant Protect Sci 48(2): 75-80.

Sambrook J, Fritsch EF and Maniatis T. 1989. Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Press, Cold Spring Harbor, New York.

Seetharaman, K., Whitehead, E., Keller, N. P, Waniska, R.D., Rooney, L.W. 1997. In vitro activity of sorghum seed antifungal proteins against grain mold phytopathogens. J. Agric. Food Chem. 45, 3666e3671.

Sharma, R., Rao, V. P., Upadhyaya, H.D., Gopal R.V., And Thakur, R.P. 2010. Resistance To Grain Mold and Downy Mildew in A Mini-Core Collection of Sorghum Germplasm. Plant Disease, 94(4): 439-444.

Shirsath, L. P., Patil, S. P. and Patil, U. K. 2018. Incidence of leaf spot disease on cotton caused by *Curvularia verruculosa* and role of its hydrolytic enzymes in pathogenesis. Physiology and Molecular Biology of Plants,.24(4): 711–714.

Singh SD, Bandyopadhyay R. 2000. Grain Mold. pp. 38-40. In: Compendium of Sorghum iseases, A. rederisen, dod, eds.). The American Phytopathological Society, St. Paul, MN, USA. pp. 38-40.

Sivanesan, A. 1987. *Graminicolous* species of *Bipolaris, Curvularia, Drechslera, Exsorohilumand* their teleomorphs. Mycological Papers, 158, 1-259.

SPSS 25.0, 2021. SPSS 25.0 for Windows. Chicago, IL, SPSS Inc. Stefan, T., 2003. Organic Agriculture: Sustainability Market Policies. CAB Organization for Economic Cooperation and Development 95 p.

Swofford DL. 2002. PAUP*: Phylogenetic Analysis Using Parsimony (and other methods) 4Æ0 Beta. Sunderland, MA, USA: Sinauer Associates. Station, Texas, USA. 177 pp.

Tarekegn, G., McLaren, N. W., and Swart, W. J. 2006. Effects of weather variables on grain mould of sorghum in South Africa. Plant Pathol. 55: 238-245.

Waniska R. D., Venkatesha. R. T., Chandrashekar A., Krishnaveni S., Bejosano F. P., Jeoung J., Jayaraj J., Muthukrishnan S. and Liang G. H. 2001. Antifungal Proteins and Other Mechanisms in the Control of Sorghum Stalk Rot and Grain Mold. *J. Agric. Food Chem.* 2001, 49, 10, 4732-474.

White Et Al. 1990. In: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA, 1990. Page 315.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535. within Sorghum bicolor (L.) Moench lines. Ph.D. dissertation, Texas A&M University, College

Yago J I, Roh J H, Bae S do, Yoon Y N, Kim H J and Nam M H. 2011. The effect of seed borne mycoflora from sorghum and finger millet seeds on germination and disease transmission. Mycobiology39: 206-218.

الملخص العربى

التوصيف والتنوع الوراثي لعزلات *Curvularia lunata* المصاحبة لعفن حبوب الذرة الرفيعة في مصر

عبير حمدى عباس - سيد حسن عجاج

قسم بحوث أمراض الذرة والمحاصيل السكرية - معهد بحوث أمراض النباتات

مركز البحوث الزراعية - الجيزة

تعتبر نباتات الذرة الرفيعة (*Sorghum bicolor*) من المحاصيل الهامة فى المناطق القاحلة وشبه القاحلة والتى تواجه ضغوطات حيوية كبيرة خاصة عفن الحبوب المتسبب عن الاصابة بفطر *Curvularia lunata* الذى ظهر بشكل بارز كمسبب لمرض تبقع اوراق وعفن حبوب الذرة الرفيعة مما أثر بشكل كبير على جودة الحبوب وإنتاجيتها.

تهدف هذه الدراسة الى عزل وتوصيف سلالات الفطر *Curvularia lunata* من حبوب الذرة الرفعية المتحصل عليها من الاسواق المصرية، تم عزل عشرة عزلات واختبارها على صنف سورجم جيزة ١٥ والذى أظهر مدى واسع من الشدة المرضية تراوحت بين 22.67 الى 66.68 %. تم التحقق من تعريف العزلات على أنها *Curvularia lunata* بواسطة التوصيفات المورفولوجية وبشجرة التقارب مع الفطريات الأخرى. وقد كشف التحليل الوراثى ببادئات ال RAPD على وجود اختلافات وراثية كبيرة بين العزلات. قسم التحليل الوراثى العزلات الى مجموعتين رئيسيتين مع تقارب التشابه الوراثى داخل كل مجموعة ومع ذلك أظهر التوصيف ببادءات ال RAPD أن هناك 38 قطعة من الحمض النووى بتعددية أشكال بنسبة 93.05 % مما يشير الى التنوع الوراثى الكبير بين العزلات مما يعطى العزلات قدرة عالية على التكيف كمسبب مرضى فى الظروف المختلفة ، تمدنا هذة النتائج برؤية مستقبلية فى تربية أصناف ذرة رفيعة مقاومة والتأكد من تطبيق استراتيجية مكافحة متكاملة للتخلص من عفن الحبوب المؤثر على انتاج السورجم.