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Molecular Characterization of Fungi in Stored Melon Seeds from South-West Nigeria

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Abstract

Stored melon seeds are infested by fungi which cause loss of quality and consequently of the seeds at various stages of deterioration. Field pests of this important seed have been numerously enumerated and conventional methods have been used to determine the identity of the pathogens. In addition to previously used techniques, this study was carried out to identify fungal pathogens of stored melon seeds and assess information provided by the phylogenetic relationships. Discoloured and mouldy seeds from four markets each in four towns in each of the six states were plated directly on freshly prepared agar. Cultural, microscopic and molecular characteristics of purified isolates were determined. One hundred and eight fungal isolates were obtained and identified as strains of *C. lunata, A. flavus, A. oryzae, C. geniculata, F. equiseti, N. sitophila, L. pseudotheobromae and P. simplicissimum*.

Keywords: Fungi, Melon seed, Nigeria, Molecular characterization.

Introduction

South West Nigeria is a geopolitical region consisting of six states mostly having tropical rainforest that supports growth of melon. Melon (*Citrullus lanatus*) is a widely cultivated and consumed oil seed crop in West Africa [1]. It is an important crop plant and vegetable in Nigeria, cultivated for its edible nutrient-rich seeds popularly called "Egusi" [2]. Melon seeds are of different varieties which include: *Citrullus lanatus, Colocynthis citrullus and Citrullus vulgaris*. The seeds are usually small, flat and oval containing a white cotyledon in a thin walled shell with a thick ring around the edge [3]. These seeds which are high in protein (34.86%) and oil (42.29%) also contains minerals such as Sodium (162.76ppm), Potassium (8.28%) and Calcium (1.49%) [4] are used as condiment in enriching the taste and appearance of local stew [2] as well as being applied for many other uses that include the extraction of its oil and the preparation of snacks and soup thickeners [5-7].

Despite the importance of melon seeds and the nutrients present, it has been reported as a disease reservoir [8] due to infection by field and storage pathogens which secrete mycotoxins, cause seed discolouration, decrease nutritive value, increase fatty acid and peroxide values and decrease seed germination [9,10].

As it is currently available, the sequence of the conserved region in the pathogen's genome can assist in their proper identification while providing information to understand variations and develop effective management strategies.

Materials and Methods

Sample collection

Melon seeds were randomly collected from four different markets in four towns in each state of the South Western geopolitical region

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of Nigeria. The seeds from each market were carefully subjected to scrutiny and the discolored, visibly mouldy and physically distorted seeds were separated.

Isolation and identification of fungi from melon seeds

Twenty seeds from the separated samples from each market were randomly selected and disinfected by rinsing in 1% sodium hypochlorite solution (1% NaOCl) for one minute, followed by successive rinses in distilled water. Eight seeds were plated in Petri-dishes containing Potato Dextrose Agar (PDA) to which 0.3% chloramphenicol was added [11]. The plates were incubated for 5-7 days at 28±2°C. Representative colonies of the fungi which appeared on each plate were subcultured until a pure culture of each isolates were obtained. The cultural and morphological characteristics were determined and compared with a standard reference [12].

Molecular characterization

This was done using standard techniques involving Extraction of DNA, Polymerase Chain Reaction using forward primer ITS-1 and reverse primer ITS-4 and sequencing. The sequences obtained were compared with the database of nucleotides in the Genbank and a phylogenetic tree was plot.

Results and Discussion

The identity of the isolates which was determined by molecular characterization shows that they bore similarities to strains of isolates in the *Aspergillus, Curvularia, Fusarium, Neurospora, Lasidioplodia* and *Penicillium* genera except for three that were 100% similar to *A. oryzae* TF7, *Curvularia lunata* ZCL3 and *Penicillium simplicissimum* NPF-5. Also found in the stored melon seeds were other species of Aspergillus including *A. oryzae* and *A. niger*. However, due to the ubiquity of *A. niger* and *Rhizopus oryza*e confirmed by its isolation in previous researches, its presence was only noted and not used in further investigations (Figure 1-2) [13].

The relatively balanced distribution of nutrients in the seed makes it an ideal substrate for growth and metabolism of various fungi. Though with a high protein content (including essential amino acids), it is known to also contain ash (minerals), carbohydrate, vitamins and fibre [14] in addition to the low moisture content which is preferable for the formation of hyphae, mycelium and spores by most field and storage moulds (Table 1).

Though sample B was identified as *C. lunata*, the phylogenetic tree shows a close relationship as well as a common ancestry with *Cochliobolus* species. Previous findings have shown that *Cochliobolus* are teleomorphs of Curvularia [15]. Isolates D and M which are members of the genus *Curvularia* were also found to be closely related bearing similar ancestry to isolate B and the teleomorph to this genus.

Furthermore, while all the isolates have been found to infect or colonise various plant parts, some representatives of *Curvularia* genus have been isolated specifically from melon seeds. The evolutionary history as shown by the phylogenetic tree in Figure 1 also suggests a close relationship between *A. oryzae* and *A. flavus* isolated as well as other similar members of the same species. This is not novel since it has been previously established that there exists a homology (about 99.5%) in the genome of both species hence suggesting their descent from the same progenitor (probably a strain of *A. flavus*) [16]. Furthermore, Kehinde [17] investigated the field pests of melon and of the

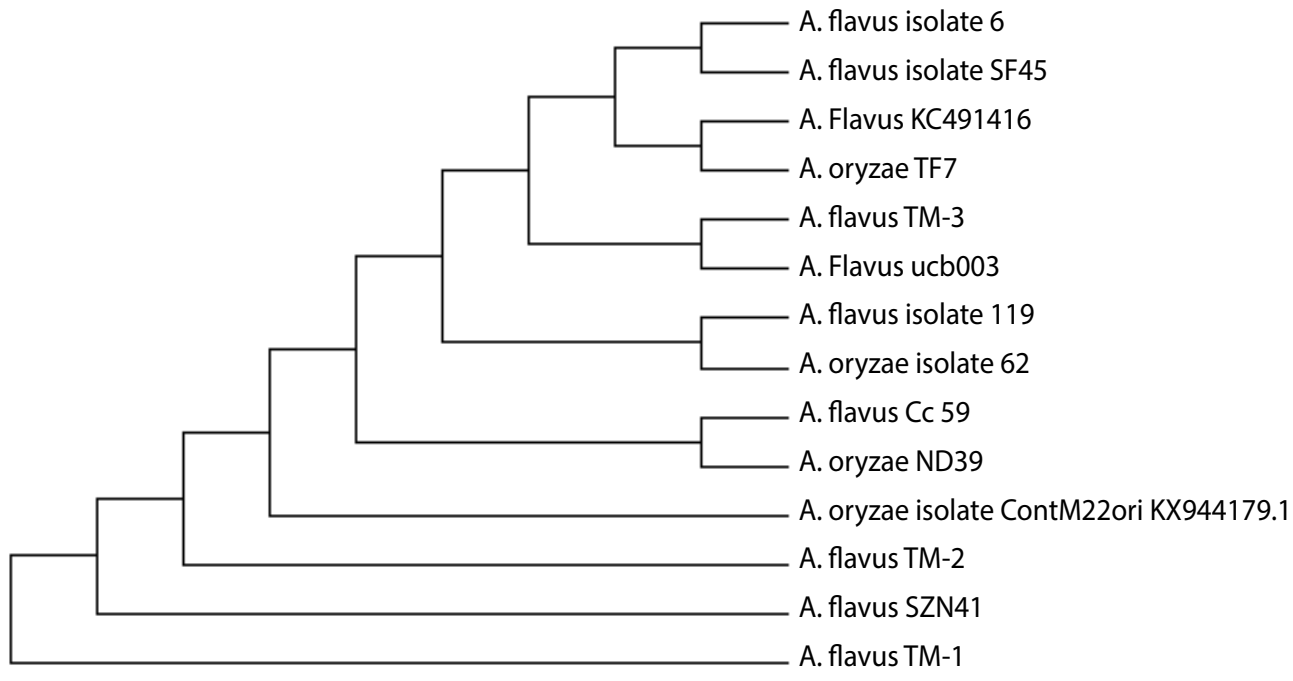

Figure 1: Phylogenetic tree showing some related isolates from stored melon seeds.

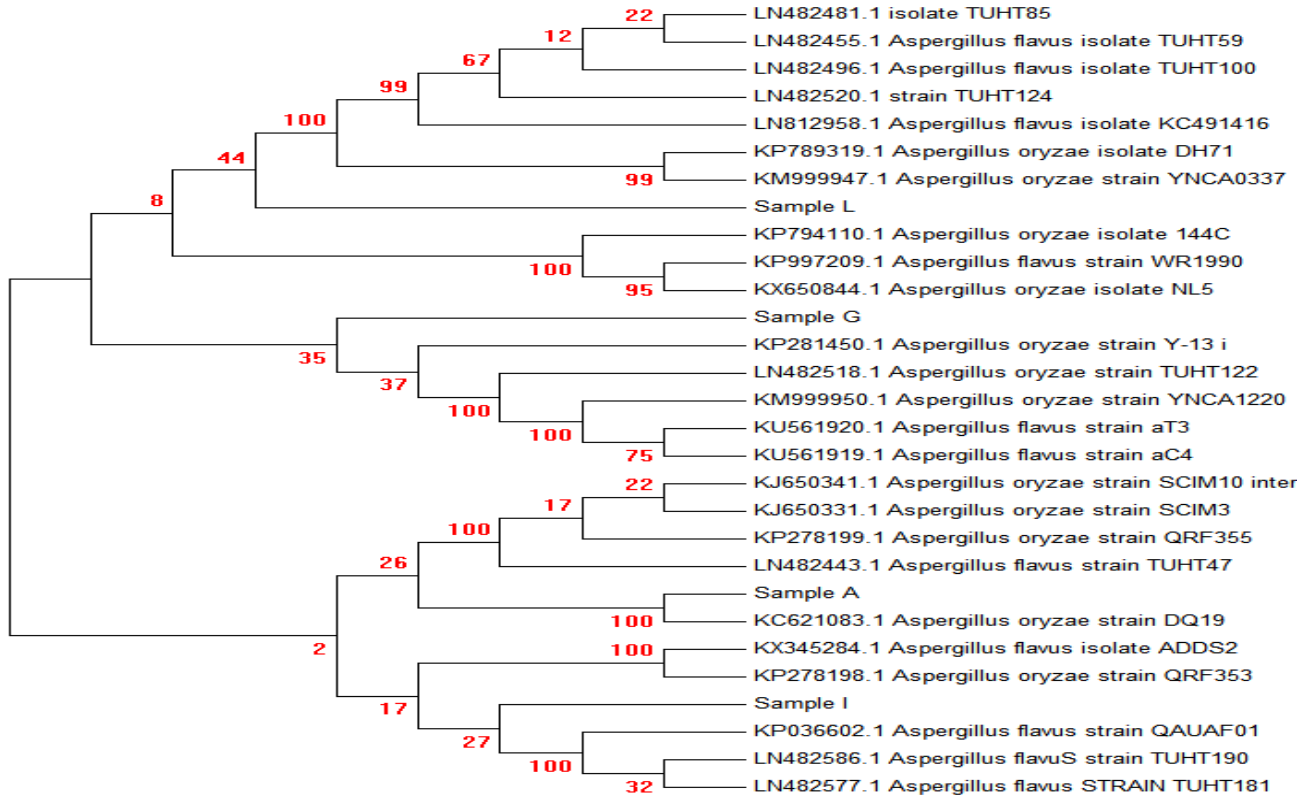

Figure 2: Phylogenetic tree showing some related isolates from stored melon seeds.

Table 1: Identification of isolates obtained from stored melon seeds.

Sample ID	Identity	Assigned Accession Number	Closest Relative	Similarity (%)
A	*Aspergillus flavus* TM-1	MH716398	*Aspergillus flavus* SZN41	94
B	*Curvularia lunata* TM-1	MH716399	*Curvularia lunata* UM296	95
D	*Curvularia lunata*		*Curvularia lunata* ZCL3	100
E	*Neurospora sitophila* TM-1	MH716400	*Neurospora sitophila* M21	97
G	*Aspergillus flavus* TM-2	MH716401	*Aspergillus flavus*	98
I	*Aspergillus oryzae*		*Aspergillus oryzae* TF7	100
L	*Aspergillus flavus* TM-3	MH716402	*Aspergillus flavus* SF45	99
M	*Curvularia geniculata* TM-1	MH716403	*Curvularia geniculata* CML3602	97
P	*Fusarium equiseti* TM-1	MH716404	*Fusarium equiseti* CGAJ-81	94
T	*Lasidioplodia pseudotheobromae* TM-1	MH716405	*Lasidioplodia pseudotheobromae* UY1356	95
U	*Penicillium simplicissimum*		*Penicillium simplicissimum* NPF-5	100

11 isolates obtained from different plant parts in that study, only *A. flavus* was found in storage during this study though representatives of *Fusarium* species were also isolated.

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