

Detection and determination of pathogenicity of seed-borne fungi in maize (*Zea mays* L.) varieties

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Abstract

A component plating study was carried out to detect seed mycoflora of four maize varieties (SUWAN IESS, DMR ESR, ACR 97 STR, ACR 97 TZLC) and the pathogenicity of fungal isolates were determined. The agar plate method was used in isolation of moulds from seed coats, cotyledons and embryos of seeds on Potato Dextrose Agar medium while the pathogenicity was determined by the seedling symptom test. *Aspergillus* spp were isolated from seeds of the four maize varieties while *Penicillium* sp., *Cladosporium* sp., *Fusarium* spp were encountered in three of the maize varieties. Seeds of the variety DMR ESR had the highest number of fungal isolates. The major site of infection in all the maize varieties was the cotyledon with 73% infection in SUWAN IESS, 60% in DMR ESR, 50% in ACR 97 STR and 75% in ACR 97 TZLC. Embryo components of seeds from the four varieties were all infected by fungi. A considerable reduction in percentage germination of seeds of the four varieties observed in the study was attributed to susceptibility of the embryos to infection. The maize variety ACR97 TZLC is less susceptible to fungal infection than other varieties. Disease symptoms in seedlings raised from seeds infected with the fungal isolates ranged from chlorotic leaves and weak stands to reduced root system. Cellulase and Pectinase activities were observed in two of the fungal pathogens; *Aspergillus flavus* and *Penicillium* sp. It was inferred that the activities of these cell wall degrading enzymes contributed to the virulence of the pathogens. This work highlights the importance of seed health testing in preventing spread of fungal pathogens on the field as relatively healthy looking seeds were found to have deep seated infections.

Key words: Seed mycoflora, maize, pectinase, cellulase.

Introduction

Maize is the third important cereal crop in the world (Rahman et al., 2008). In Nigeria, the situation is the same with the crop serving as an important staple food consumed in its various forms. Production of the crop is on the increase especially with its introduction to the Northern Guinea Savannah (Olatunji et al., 2005). The domestic supply however has not been able to meet up with the demand. This is probably due to slow rate of development of improved high yielding varieties and more importantly the effect of diseases. A total of about 112 diseases are known to affect the crop (USDA, 1960) and among them more than 70 are seed borne. Richardson (1990) identified some of the important seed borne diseases of maize to include leaf spot, leaf blight, collar rot, kernel rot, scutellum rot, seedling blight, anthracnose and head smut. Fakir (2001) also named 11 seed borne diseases of maize in Bangladesh including black band, anthracnose and stem rot caused by *Boeromyces* *therobromae*, *Colletotrichum* *corchori* and *Macrophoma* *phaseolina* respectively. In the Southern Guinea Savanna agro-ecology of Nigeria, there is dearth of information on seed-borne infection of maize and their significance in reduction of maize yield. This work thus examines the seed mycoflora of the popularly cultivated maize varieties in Southern Guinea Savanna zone of Nigeria and evaluates the pathogenic effects of the fungi on maize seedlings.

Materials and methods

The seeds of maize varieties SWAN IESR, DMR ESR, ACR 97 STR and ACR 97 TZLC used were obtained from the seed council of the Federal Ministry of Agriculture and Water Resources in Ilorin. The seeds were sorted to separate healthy looking from physically damaged ones. Seed borne fungi were detected using agar plate method (ISTA, 1996.) with slight modifications. The seeds were pre-treated with 0.5% NaOCl solution for five minutes followed by rinsing in sterile water and were later plated on Potato Dextrose Agar (PDA) amended with 1% streptomycin sulphate in Petri dishes (9cm diameter) at the rate of ten seeds per plate. Ten replicate plates were used and incubated for seven days under twelve hours alternating cycles of Near Ultra Violet (NUV) light and darkness at 22°C±2°C. Incubated seeds were examined for mycelia growth and the fungal colonies were counted. Pure cultures were prepared from the designated colonies observed by transferring hyphae or spores aseptically onto sterile media with alcohol-flamed inoculating needle in an inoculating chamber. These were incubated for 8 days at 28±2°C. Identification of fungi was based on their growth characteristics on PDA and microscopic examination of conidia following standard references like Malone and Maskett (1997); Kulwant *et al.*, (1991).

The seeds were also separated into component parts; testa, cotyledons and embryo. The components were later surface sterilized as described above and plated on PDA in petri dishes and incubated for eight days at 28°C (Saad *et al.*, 1988). After incubation, seeds were examined for growth. Isolated fungi were identified as earlier described.

For test of pathogenicity, spore and mycelia suspensions were prepared from the fungal isolates. Spore suspension was prepared for sporulating organisms with the spore concentration adjusted to 5×10^5 spore/ml using haemocytometer (Shorvan *et al.*, 2008). Mycelia suspension was prepared for isolates that do not sporulate easily by punching 5mm disc of fresh growing mycelia with cork borer. Five mycelial discs were added to 150ml of sterile water and blended in a waring blender.

Effect of inocula on germination of maize seeds and seedling health was studied by shaking surface sterilized seeds with the inocula for approximately one hour with wrist action mechanical shaker in a corked conical flask. The seeds were later left to stand in the inocula for 8-10 hours. They were then collected with and allowed to dry on sterile paper towels. Inoculated seeds were sown in plastic pots (30cm×25cm×12cm) filled with sterile soil. Wetting was done with sterile water and the pots were incubated at room temperature for 6-10 days. The setup was examined for germination and seedling symptoms. Data collected for germination studies were subjected to analysis of variance using Genstat532 statistical package. Significant means were separated by Duncan's multiple range test at 5% probability level.

Two of the virulent fungi isolated in the study were selected for the assay of cell wall degrading enzymes activities. The selected organisms were cultured on PDA slants for 3 days at 28±2°C and later washed with sterile water to dislodge the spores which were then used to prepare enzyme filtrate. Fifty grams of ground and sterilized maize seeds (the varieties used) was moistened with 50ml spore suspension (5×10^5 spore/ml) of the virulent fungi separately and the mixture incubated for 3-5 days at about 28±2°C. Sterile water (150ml) was later added in each case and the mixture agitated for 30 minutes with mechanical shaker. The mixture was filtered using sterile Whatman No.1 filter paper. For the assay of cell wall degrading enzymes, viscometric method described by Imdo (1961) was used. In this method, the reaction mixture contained substrate- 15ml carboxymethylcellulose (CMC) (1% w/v) in citrate phosphate buffer (pH 5.5) and 15ml enzyme filtrate for cellulase assay. For pectinase assay, the mixture contained substrate- 15ml pectin (2% w/v) in citrate phosphate buffer (pH 5.5) and 5ml of the enzyme filtrate. Measurements were made in glass viscometer after the mixtures have been incubated for 2 hours at 30°C. The enzyme activities were expressed as percentage viscosity change. The reducing rate of viscosity was calculated using the equation;

$$A = \frac{V_0 - V_1}{V_0 - V_2} \times 100 \quad (1)$$

V_0 = flow time in seconds of substrate + inactive enzyme

V_1 = flow time in seconds of substrate + active enzyme

V_2 = flow time in seconds of solvent (water) + inactive enzyme

One unit of viscosity reducing activity is defined as the quantity of enzyme necessary for 50% viscosity change of 20ml of the reaction mixture at 30°C.

Results and discussion

Seven fungal species were isolated from the four maize varieties used in the study (Table 1).

Table 1: Population of fungi isolated from maize seeds on Potato Dextrose Agar.

Maize Variety	Fungal isolate	Fungal count Mean $\pm$ SE
DMR ESR	<i>Penicillium</i> sp.	0.29 $\pm$ 0.88
	<i>Cladosporium</i> sp.	0.19 $\pm$ 0.58
	<i>Fusarium solani</i>	0.08 $\pm$ 0.33
	<i>Fusarium oxysporum</i>	0.29 $\pm$ 0.33
	<i>Aspergillus niger</i>	0.15 $\pm$ 0.33
ACR 97 STR	<i>Penicillium</i> sp.	0.31 $\pm$ 0.33
	<i>Cladosporium</i> sp.	0.27 $\pm$ 0.33
	<i>Aspergillus flavus</i>	0.23 $\pm$ 0.00
	<i>Aspergillus niger</i>	0.19 $\pm$ 0.33
SUWANESR	<i>Fusarium solani</i>	0.18 $\pm$ 0.00
	<i>Aspergillus flavus</i>	0.36 $\pm$ 0.88
	<i>Aspergillus niger</i>	0.45 $\pm$ 0.33
ACR97TZLC	<i>Penicillium</i> sp.	0.28 $\pm$ 0.33
	<i>Cladosporium</i> sp.	0.16 $\pm$ 0.33
	<i>Fusarium solani</i>	0.24 $\pm$ 0.58
	<i>Aspergillus flavus</i>	0.32 $\pm$ 0.62

Values are means from 10 readings.

The fungi appeared clearly on the culture plates between 3-7 days of incubation. Maize variety DMR ESR had the highest number of isolates while SUWAN1-ESR had the lowest number of isolates. The use of agar plate incubation technique was efficient for the isolation of seed-borne mycoflora of maize. The efficacy of this isolation technique was also reported by Ishtat and Dawar (2009). *Aspergillus* spp. were common to all maize varieties used. The component plating study revealed the cotyledon as the main site of infection of the maize seeds with 73%, 60%, 50% and 75% infection in SUWAN 1 ESR, DMR ESR, ACR-97 STR and ACR-97 TZLC respectively (Fig. 1). The seed coat had the lowest percentage occurrence of fungal isolates. Nasreen (2003) showed a reduction in the incidence of superficial, fast growing saprophytic and pathogenic fungi on seeds that were surface sterilized with sodium hypochlorite. Surface disinfection of seeds prior to health testing is therefore a possibility of discouraging the growth of ordinary seed contaminants. Shahnaiz *et al.* (2007) reported a reduced incidence of *Aspergillus* species with surface sterilization and high number of fungi in seed coat and cotyledon of chickpea seeds.

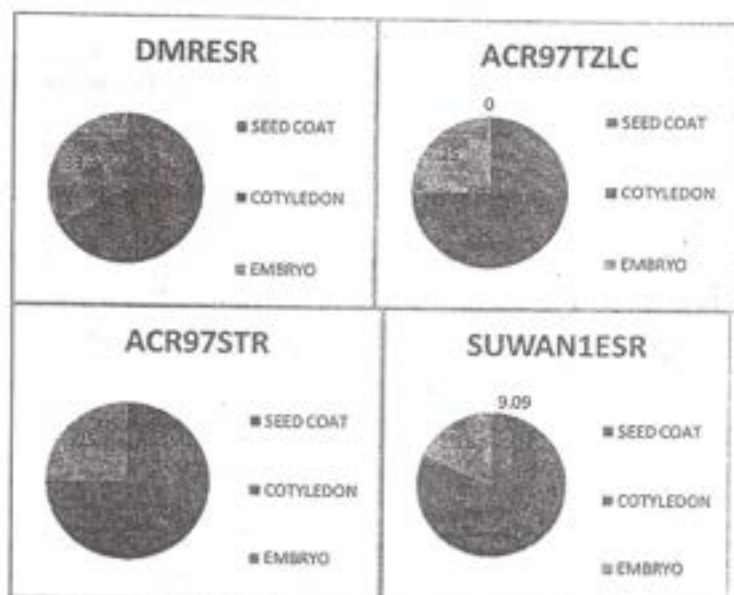


Fig. 1. Percent infection of seed components of different maize varieties plated on Potato Dextrose Agar.

The embryos of the maize seeds for all varieties studied were infected by fungi. *Aspergillus* spp. were isolated from the embryo of SUWAN1 ESR, ACR-97 STR and ACR-97 TZLC while *Cladosporium* sp. was isolated from the embryo of DMR-ESR (Table 2).

Table 2. Incidence of moulds in maize seed components plated on Potato dextrose Agar

Maize Variety	Seed Component	Fungal isolates					
		<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>	<i>Cladosporium</i> sp.	<i>Fusarium oxysporum</i>	<i>Fusarium solani</i>	<i>Penicillium</i> sp.
DMR ESR	Seed Coat	-	+	-	-	-	-
	Cotyledon	-	-	+	+	-	-
	Embryo	-	-	+	-	-	-
SUWANI ESR	Seed coat	-	+	+	-	-	-
	Cotyledon	-	-	-	-	+	-
	Embryo	+	-	-	-	-	-
ACR97STR	Seed coat	-	-	-	+	+	-
	Cotyledon	-	-	-	-	-	+
	Embryo	-	+	-	-	-	-
ACR97TZLC	Seed Coat	-	-	-	-	-	-
	Cotyledon	-	-	+	-	-	-
	Embryo	-	+	-	-	-	-

The inoculated fungal isolates reduced percentage seed germination of the four maize varieties considerably compared with the control (Table 3).

Table 3. Effect of fungal infections on germination of maize seeds

Fungal inoculum	% Germination of seeds for different maize varieties				
	DMR ESR	ACR97STR	SUWANI ESR	ACR97TZLC	GRAND MEAN
<i>Aspergillus flavus</i>	43.3	43.3	40.0	57.0	45.9 ^a
<i>Aspergillus niger</i>	40.0	47.0	43.0	47.0	44.3 ^{bc}
<i>Cladosporium</i> sp.	23.3	39.0	57.0	43.0	40.6 ^{bc}
<i>Fusarium oxysporum</i>	20.0	47.0	43.3	47.0	39.3 ^{bc}
<i>Fusarium solani</i>	17.0	17.0	43.3	60.0	34.3 ^{bc}
<i>Penicillium</i> sp.	30	23.3	33.3	40.0	31.7 ^a
Control (uninoculated)	100	93.3	83.3	93.3	92.5 ^a
Grand mean	39.1 ^c	44.0 ^{bc}	49.1 ^{ab}	55.4 ^a	

Means followed by the same alphabet along rows and columns are not significantly different by DMRT at 1% probability. SED for isolates=12.36; SED for maize variety=9.34

Maize variety, DMR ESR was more susceptible to fungal attack than other varieties as shown by the high reduction in germinability. The germinability of seeds of the different varieties infected with fungal pathogens was in the order ACR97 TZLC > SUWANI ESR > ACR97STR > DMR ESR. Ability of some of the fungal isolates to infect the embryo would affect the germinability of the seeds adversely. The location of pathogen in seeds affects viability as well as control of seed borne infections. The deeper the location, the more difficult it is to control (Dalbir and Mathur, 2004). Infection of the cotyledon and embryo probably lead to reduction in food reserves in the seeds. Kashinath and Rama (2002) reported gradual loss of carbohydrate content in seeds of maize, groundnut and soybean with mould infection. An initial protein loss was also reported. All the fungal isolates in this study induced disease symptoms on the maize seedlings. The symptoms observed ranged from chlorosis, necrotic spots on leaves, stems and roots while the uninoculated control remained healthy (Table 4). Assay for the production of cell wall degrading enzymes two of the pathogens showed that cellulases and pectinases were produced (Fig.2). The enzyme preparation from *Penicillium* sp. had a

higher cellulase activity (73.15 VRU/ml) than that of *A.niger*. Pectinase activity was however found to be higher in enzyme preparation of *A.niger* (55.56 VRU/ml) than that of *Penicillium sp.* Many species of *Aspergillus* and *Penicillium* are known to be pectolytic, (Fawole and Odunfa, 1992).

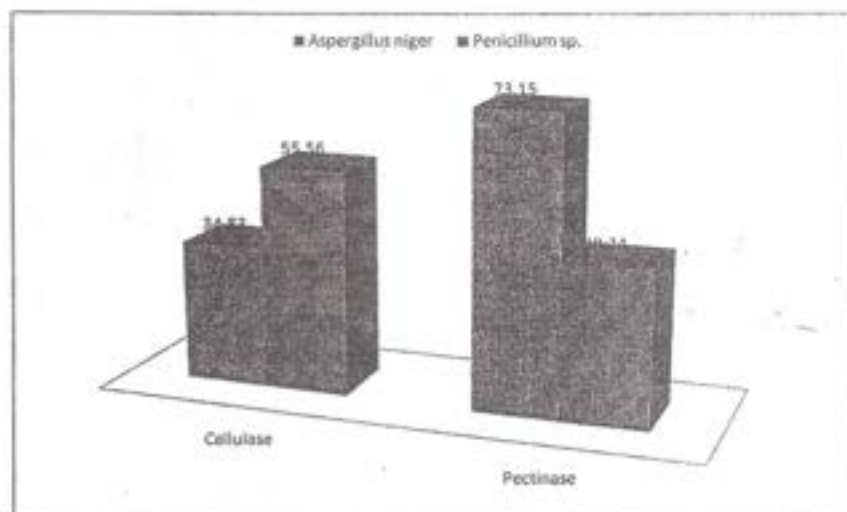


Fig. 2. Pectolytic and Cellulolytic activities (VRU/ml) of enzyme preparations from *Aspergillus niger* and *Penicillium sp.*

Table 4: Pathogenic effects observed on maize seedlings raised from seeds infected with fungal isolates

Fungal isolates	Maize variety			
	SUWANIESR	DMR ESR	ACR97STR	ACR97TZLC
<i>Aspergillus flavus</i>	Seed rot	Seed rot and damaged roots	Seed rot and weak seedlings	Seed rot and stunted growth
<i>Aspergillus niger</i>	Seed rot and stunted seedlings	Seed rot and stunted growth	Seed rot, decay of root system and yellowing of leaves	Seed rot, reduced root system and stunted growth
<i>Cladosporium</i> sp.	Seed rot and yellowing of leaves	Seed rot, chlorotic and crinkled leaves	Seed rot, dark spots on cotyledons	Root damage, drying of first leaves
<i>Fusarium cryptotum</i>	Seed rot, unhealthy seedlings with damaged roots and chlorotic leaves	Seed rot, unhealthy seedlings with damaged and chlorotic leaves	Seed rot, yellowing of leaves and reduced leaves	Seed rot
<i>Fusarium rolandi</i>	Seed rot, reduced roots, yellowing of leaves	Seed rot, weak seedlings, stunted growth and chlorotic leaves	Seed rot and stunted seedlings	Seed rot, and weak seedlings
<i>Penicillium</i> sp.	Seed rot and damaged plumule	Seed rot and weak seedlings	Seed rot	Seed rot, damaged roots and plumule
Control	Healthy seedlings	Healthy seedlings	Healthy seedlings	Healthy seedlings

The ability of these fungi to produce cellulases and pectinases probably explains their virulence as these enzymes would facilitate their penetration into the seeds to cause deep seated infection and the symptoms observed in the seedlings.

Conclusion

Fungal infection of maize seeds has a major impact on seed germination and carry-over diseases as shown by seedling symptom tests. Knowledge of the major site of infection in the seeds will be useful in the determination of appropriate control measures. The maize variety ACR97 TZLC is less susceptible to fungal infection than other varieties.

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