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Effect of harvest stage and drying methods on germination and seed-borne fungi of maize (*Zea mays* L.) in South West Nigeria

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Germination of seed and infection by seed-borne fungi of two maize varieties DMRLSR-W and DMRLSR-Y as affected by stage of harvest and method of drying were studied in the growing seasons of year 2002 and 2003 at the Institute of Agricultural Research and Training, Moor Plantation, Ibadan, Nigeria. The experiment was a split-plot arranged in a randomized complete block design with four replications. Ears were harvested at 30, 35, 40, and 45 days after tasselling (DAT). Extracted seeds were dried to 13-14% moisture content (i) in shade, (ii) in sun, and (iii) artificially at 45°C in a Thermax batch type seed drier. "Initial" and "final" germination tests were conducted immediately after harvest and drying, respectively. Significant higher percentage germination was associated with seeds from cobs harvested 35 DAT which were sun dried. The highest 100-seed weight was recorded in seeds from cobs harvested 45 DAT irrespective of drying method. Associated fungi were isolated by plating seeds on potato dextrose agar. Results indicated seeds from ears harvested at 30 and 35 DAT irrespective of drying method were not infected with Fusarium moniliforme, Fusarium graminearium, Botryodiplodia theobromae and Drechslera maydis. However, seed germination was significantly lower for seeds from ears harvested at 30 DAT. Aspergillus sp. were isolated from all the seeds irrespective of stage of harvest and drying method. Penicillium sp. was not isolated from any of the seeds dried artificially. Significant higher percentage germination was associated with seeds from cobs harvested 35 DAT which were sun dried. Maximum 100-seed weight was recorded in seeds from ears harvested 45 DAT irrespective of drying method.

Key words: Seed-borne, fungi, harvest, stage, drying, germination, *Zea mays*.

INTRODUCTION

The seed is the nucleus of farmer's production activities hence its quality should be guaranteed at all times (Owolade et al., 2001). About 90% of all the food crops grown in the world are propagated by seeds. In any crop production systems, good quality seed inspires the confidence of farmers, because all other inputs will merely assist the seed to produce optimally. Germination percentages and purity are the two factors given priority

in seed certification, whereas stage of seed maturity for harvest and method of drying are among the major factors deciding seed quality (Roberts, 1981). Maize (*Zea mays* L.) quality is often reduced because of drying injury, although the causes and impairment mechanisms are poorly understood. However, the impairment of lip body alignment along the plasma membrane during artificial drying of maize has been associated with decrease in germination and vigor (Cordova-Tellez and Burris, 2002). Drying is an expensive and energy demanding operation. Furthermore, both the velocity of the drying and the maximal temperature of the grain have to be controlled to prevent a possible reduction of germination.

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Seed-borne pathogens, of which, fungi accounts for 75% of reported cases of association have also been found to cause maximum damage such as abortion, rot, necrosis, discolouration, and reduced germination and vigour (Shetty, 1988). Fungi that produce toxins in food are classified into field fungi and storage fungi based on their ecological requirement for growth (Bankole, 1994). The first group requires grain moisture above 20% in cereals and often causes ear rot and toxin production before harvest. Fusarium sp. is the most important field fungi of maize worldwide and is known to produce over 100 secondary metabolites that can adversely affect human and animal health (Visconti, 2000). Fusarium moniliforme has been found to be most wide spread and most frequent in preharvest and stored maize in Nigeria (Essien, 2000). Aspergillus flavus, A. parasiticus and A. nominus have also been reported to be wide spread in Nigeria on seeds and chips of several crops. Apart from the fact that these fundi can cause diseases on the field. they are also known for the production of toxic metabolites. Bankole and Adebanjo (2003) reported that inhabitant of sub-Sahara Africa are experiencing heavy dietary exposure to food borne mycotoxins particularly fumonisins produced by F. moniliforme and aflatoxins from infection by Aspergillus sp. Early harvest and rapid drying have been advocated as a means of reducing the incidence of seed-borne pathogen and consequently reducing the level of toxic metabolites production. Therefore, the aim of this study is to investigate the optimal stage of maturity and drying method best suited for a successful and cost effective production of biologically viable uninfected maize seed.

MATERIALS AND METHODS

Plant material, seed production and analysis

The seeds of two varieties of maize, DMRLSR-W and DMRLSR-Y, which are popular among the farmers in the south west Nigeria were collected from the seed store of the Institute of Agricultural and Training, Ibadan. Field experiments were conducted during the growing seasons of 2002 and 2003 at the Institute. The site is located at latitude 7° 31 N and longitude 3° 45 E and 210 m above sea level in the forest-savanna transition agro-ecological zone of Nigeria (IAR&T, 2000). The field was laid out in a spit plot arranged in a randomized complete block design with stage of harvest as the main plot and method of drying as sub-plot treatment replicated four times. The maize cobs were harvested at 30 days after tasselling (DAT) (when all the husk leaves were green), 35 DAT (when nine of the husk leaves were light green), 40 DAT (four of the husk leaves light green), and 45 DAT (when all the husk leaves were dry). Some seeds were extracted to determine initial germination and moisture content. The remaining cobs were dried to 13.5 to 14.0% moisture (i) in shade, (This was made of wood/bamboo on raised platforms or with metal roofing with adequate ventilation from all sides. The sides were covered wire mesh to prevent rodents; (ii) in sun; and (iii) artificially in Thermax batch type seed drier at 45 ℃. Random seed sample from dried seeds were used to estimate germination percentage, '100-seed' weight and percentage incidence of seedborne fungi.

Isolation and identification of seed-associated fungi

Seed sample of each cultivar per treatment were surface-sterilized in 2% sodium hypochlorite solution for 5 min and then rinsed in three changes of sterile distilled water prior to plating. Four hundred seeds of each cultivar from all the treatments were plated in four replicates of 100 seeds each. Five seeds were plated in each Petri dish containing 10 ml of potato dextrose agar (PDA). These were then incubated at 27 ± 2°C under alternating cycles of 12 h near violet (NUV) light and darkness. On the 8th day of incubation, each seed was examined thoroughly under a 12-60X MS binocular stereo-microscope and a compound light microscope. Fungi were identified on the basis of growth characteristics (Barnett and Hunter, 1972). Pure cultures and slide preparations were compared with standards obtained from the plant pathology laboratory of International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The data were subjected to analysis of variance (ANOVA) without transformation, and means were separated using Duncan's multiple range test (DMRT).

RESULTS

The result in Table 1 show that the seeds harvested at 30 DAT with initial moisture content of 49.5 to 50.0% did not germinate when planted immediately after harvest. The lowest initial seed moisture content of between 14.50 and 15.00% with corresponding highest germination percentage of 82 to 98% were obtained in seeds harvested 45 DAT. The seeds from the ears harvested 35 DAT and 40 DAT which contained 38.5 to 39.5% and 24.30 to 24.50% moisture, respectively, increased from between 42.3 and 54.8% pre-drying to between 62.0 and 98% after drying, irrespective of drying method. Seeds harvested 35 DAT and 45 DAT and sun dried as well as seeds harvested 40 DAT and dried under the shade recorded the highest percentage germination of 98%.

Fusarium moniliforme Sheldon, Fusarium graminearium Schualbe, Botrydiplodia thebromae Pat, Drechslera maydis Nisicado, Penicillium spp. and Aspergillus spp. were the most frequently isolated phytopathogens from the seeds of the two varieties of maize used in this study (Tables 2 to 4). The results in Tables 2 and 3 shows that seeds from ears harvested 30 and 35 DAT were completely free of infection by F. moniliforme, F. graminearium, B. thebromae and D. maydis, irrespective of drying methods, varieties and year. Seeds from cobs harvested 40 and 45 DAT were infected with F. moniliforme and the percentage incidence ranged between 0.5 and 80.5%. The infection counts of F. graminearium were generally low on seeds from seeds from ears harvested 40 and 45 DAT. This ranged from 0.5 and 11.0% incidence across the varieties, method of drying and year. The seeds of the ears harvested 30 and 35 DAT irrespective of the drying method and variety were not associated with B. theobromae and D. maydis (Table 3). The percentage incidence of B. theobromae (20.5%) and D. maydis (38.5%) were highest on the seeds from cobs of variety DMRLSR-W harvested at 45 DAT and dried under the shade in year 2002. However, the seeds of DMRLSR

Table 1. Effect of the stage of harvest and drying methods on maize seed germination in year 2002 and 2003.

Stage of harvest/	AIMC*	AFMC**	ING***	C	Germination % after drying		
method of drying				DMRLSR-W DMRLSR-Y			SR-Y
				2002	2003	2002	2003
			30 DAT				
Shade drying	49.50	13.20	0.00	84.0	69.0	66.0	67.0
Sun drying	50.00	13.50	0.00	79.0	80.0	72.0	65.5
Artificial drying	49.50	13.00	0.00	38.0	40.0	39.0	42.0
			35 DAT				
Shade drying	38.00	13.00	49.50	93.0	93.0	96.0	93.0
Sun drying	39.50	13.20	45.00	97.0	97.0	98.0	97.0
Artificial drying	39.50	13.00	42.30	70.0	62.0	80.0	79.0
			40 DAT				
Shade drying	24.30	13.10	51.20	95.0	98.0	95.0	98.0
Sun drying	24.50	13.30	52.00	96.0	98.0	96.0	95.0
Artificial drying	24.30	13.20	54.80	82.0	89.0	80.0	79.0
45 DAT							
Shade drying	14.50	13.20	82.50	98.0	91.0	96.0	92.0
Sun drying	14.00	13.20	88.10	98.0.	92.0	98.0	90.0
Artificial drying	15.50	13.20	90.00	84.0	86.0	84.0	82.0
LSD p < 0.05				11.50	10.50	12.60	13.20

 $[\]begin{split} & \text{AIMC*} = \text{Average initial moisture content across varieties and year.} \\ & \text{AFMC**} = \text{Average final moisture content across varieties and year.} \\ & \text{ING**} = \text{Average initial germination.} \end{split}$

Table 2. Effect of stage of harvest and drying methods on the percentage incidence of Fusarium moniliforme and Fusarium graminearium in maize in year 2002 and 2003.

Stage of harvest/	Fus	Fusarium graminearium							
method of drying	DMRLES	DMRLSR-W		DMRLESR-Y		DMRLSR-W			
	2002	2003	2002	2003	2002	2003	2002	2003	
			30DAT						
Shade drying	0.0 ^d	0.0 ^d	0.0 ^d	0.0 ^e	0.0 ^b	0.0 ^c	0.0 ^c	0.0 ^d	
Sun drying	0.0 ^d	0.0 ^d	0.0 ^d	0.0 ^e	0.0 ^b	0.0 ^c	0.0 ^c	0.0 ^d	
Artificial drying	0.0 ^d	0.0 ^d	0.0 ^d	0.0 ^e	0.0 ^b	0.0°	0.0 ^c	0.0 ^d	
			35DAT						
Shade drying	38.00	13.00	49.50	93.0	93.0	96.0	93.0		
Sun drying	39.50	13.20	45.00	97.0	97.0	98.0	97.0		
Artificial drying	39.50	13.00	42.30	70.0	62.0	80.0	79.0		
	40DAT								
Shade drying	27.5 ^b	14.5°	35.5 ^a	30.0°	4.8 ^{ab}	1.5 ^c	0.0 ^c	21.0 ^a	
Sun drying	15.5°	24.0 ^b	10.5 ^c	24.5 ^d	0.5 ^b	0.5 ^c	6.0b	20.5 ^a	
Artificial drying	0.5 ^d	0.0 ^d	0.0d	0.0 ^e	0.0 ^c	0.0 ^c	4.5b	10.0 ^b	
	45DAT								
Shade drying	45.5 ^a	30.0 ^b	40.5 ^a	80.5 ^a	10.5 ^a	10.5 ^b	10.0a	8.5 ^b	
Sun drying	26.5 ^b	42.5 ^a	27.0 ^b	40.5 ^b	2.8 ^b	20.5 ^a	0.0 ^c	2.5 ^c	
Artificial drying	12.5°	10.5 ^{ab}	3.3 ^d	10.5 ^d	4.5 ^{ab}	10.0 ^b	0.0 ^c	0.0 ^d	

^{*}Each value is a mean of four replicates (100 seeds/replicate/cultivar/year).

Means followed by the same letter in the same column are not significantly different at p<0.05 using Duncan's Multiple Range Test.

Table 3. Effect of stage of harvest and drying methods on the percentage incidence of *Botrydiplodia thebromae* and *Drechslera maydis* in maize in year 2002 and 2003.

Stage of harvest/	Botrydiplodia thebromae				Drechslera maydis						
Method of drying	DMR	RLESR-Y DMRLS		SR-W DMRLES		R-Y	DMRLSR-W				
	2002	2003	2002	2003	2002	2003	2002	2003			
	30 DAT										
Shade drying	0.0°	0.0°	0.0°	0.0 ^e	0.0 ^b	0.0 ^d	0.0 ^d	0.0 ^d			
Sun drying	0.0 ^c	0.0 ^c	0.0 ^c	0.0 ^e	0.0 ^b	0.0 ^d	0.0 ^d	0.0 ^d			
Artificial drying	0.0 ^c	0.0 ^c	0.0 ^c	0.0 ^e	0.0 ^b	0.0 ^d	0.0 ^d	0.0 ^d			
			3	SDAT							
Shade drying	0.0 ^c	0.0°	0.0 ^c	0.0 ^e	0.0 ^b	0.0 ^d	0.0 ^d	0.0 ^d			
Sun drying	0.0 ^c	0.0 ^c	0.0 ^c	0.0 ^e	0.0 ^b	0.0 ^d	0.0 ^d	0.0 ^d			
Artificial drying	0.0 ^c	0.0 ^c	0.0 ^c	0.0 ^e	0.0 ^b	0.0 ^d	0.0 ^d	0.0 ^d			
	40DAT										
Shade drying	8.0 ^{bc}	10.0 ^{ab}	16.5 ^a	14.0 ^b	0.5 ^b	9.5 ^{ab}	16.2 ^b	10.5 ^b			
Sun drying	7.5 ^{bc}	13.5 ^a	15.8 ^b	10.5 ^c	4.8 ^a	8.0 ^b	17.5 ^b	10.0 ^b			
Artificial drying	2.0 ^c	0.5 ^c	12.5 ^b	0.0 ^e	0.0 ^b	0.0 ^c	0.0 ^d	0.0 ^d			
45DAT											
Shade drying	12.0 ^a	17.5 ^a	20.5 ^a	18.0 ^a	3.8 ^{ab}	18.5 ^a	28.0 ^a	38.5 ^a			
Sun drying	11.8 ^a	16.5 ^a	18.5 ^a	21.0 ^a	4.8 ^a	0.5 ^c	14.0 ^b	10.0 ^b			
Artificial drying	6.5 ^{bc}	2.5 ^c	16.5 ^a	4.5 ^d	5.0 ^a	0.0 ^c	3.5 ^c	4.0 ^c			

Each value is a mean of four replicates (100 seeds/replicate/cultivar/year).

Means followed by the same letter in the same column are not significantly different at P<0.05 using Duncan's Multiple Range Test

Table 4. Effect of stage of harvest and drying methods on the percentage incidence of *Aspergillus* spp. and *Penicillium* spp. in maize in year 2002 and 2003.

Stage of harvest/Method of		Penicillium spp						
Drying	DMRLESR-Y		DMRLSR-W		DMRLESR-Y		DMRLSR-W	
	2002	2003	2002	2003	2002	2003	2002	2003
	<u> </u>	30	DAT					
Shade drying	40.5 ^b	15.0 ^e	20.5 ^e	20.0 ^e	10.5 ^a	4.0°	10.5 ^a	18.0 ^a
Sun drying	52.5 ^{ab}	40.5 ^c	26.6 ^d	10.5 ^f	12.5 ^a	30.5 ^a	8.0 ^a	15.0 ^b
Artificial drying	48.0 ^b	51.3 ^b	25.5 ^d	20.5 ^e	0.0 ^d	0.0 ^c	0.0 ^c	0.0 ^c
		35	DAT					
Shade drying	47.5 ^b	11.5 ^e	37.5 ^c	10.5 ^f	7.5 ^b	13.5b	12.5 ^a	10.5 ^c
Sun drying	68.0 ^a	20.5 ^e	28.2 ^d	13.0 ^f	8.0 ^b	12.5b	6.0 ^{ab}	20.2 ^a
Artificial drying	46.0 ^b	0.0 ^f	28.5 ^d	8.50 ^f	0.0^{d}	0.0 ^c	0.0 ^c	0.0°
		40	DAT					
Shade drying	51.5 ^{ab}	52.0 ^b	39.5°	30.0 ^d	9.6 ^b	15.5 ^b	8.0 ^a	20.0 ^a
Sun drying	54.0 ^{ab}	68.5 ^a	62.0 ^a	50.5 ^a	2.0°	12.5 ^b	7.5 ^a	10.8 ^c
Artificial drying	66.5 ^a	60.5 ^{ab}	26.9 ^d	20.0 ^e	0.0 ^d	0.0 ^c	0.0 ^c	0.0 ^d
	•	45	DAT					
Shade drying	45.0 ^b	42.5 ^c	56.0 ^a	42.0 ^b	6.9 ^b	0.0°	7.5 ^a	0.0 ^d
Sun drying	28.0°	30.8 ^d	46.0 ^b	36.0°	8.5 ^b	10.5 ^d	5.0 ^b	16.0 ^b
Artificial drying	34.5°	16.5 ^e	18.5 ^e	0.0 ^g	0.0^{d}	0.0°	0.0 ^c	0.0 ^d

Each value is a mean of four replicates (100 seeds/replicate/cultivar/year).

Means followed by the same letter in the same column are not significantly different at P<0.05 according to Duncan's Multiple Range Test.

Stage of harvest/Method of Drying	of Drying DMRLESR-Y			DMRLSR-W		
	2002	2003	2002	2003		
	30DAT	•				
Shade drying	7.68* ^c	6.75 ^d	7.66 ^c	8.56 ^c		
Sun drying	8.61 ^c	7.68 ^{cd}	6.56 ^c	9.27 ^b		
Artificial drying	7.74 ^c	8.97 ^c	8.57 ^{bc}	8.22 ^c		
	35DAT					
Shade drying	10.39 ^b	9.67 ^c	9.50 ^b	10.24 ^b		
Sun drying	10.32 ^b	8.70 ^c	9.67 ^b	9.44 ^b		
Artificial drying	12.67 ^b	10.90 ^b	10.42 ^b	9.92 ^b		
	40DAT					
Shade drying	13.43 ^{ab}	14.70 ^a	14.75 ^a	15.32 ^a		
Sun drying	14.24 ^a	14.60 ^a	15.47 ^a	14.47 ^a		
Artificial drying	15.95 ^a	15.74 ^a	15.50 ^a	15.44 ^a		
	45DAT					
Shade drying	14.90 ^a	16.60 ^a	15.40 ^a	15.34 ^a		
Sun drying	16.13 ^a	14.75 ^a	15.50 ^a	15.44 ^a		
Artificial drying	15.37 ^a	15.80 ^a	15.40 ^a	15.62 ^a		

Table 5. Effect of stage of harvest and drying methods on 100-seed weight of maize at 15% moisture content.

-W harvested 40 DAT and artificially dried were completely free *D. maydis*, irrespective of the year. Table 4 shows that the percentage incidence of *Aspergillus* spp. was significantly influenced by the stage of harvest and drying method. The seeds from ears were infected with *Aspergillus* irrespective of variety, year, stage of harvest and drying method. The highest percentage incidence of Aspergillus spp (68.5%) was associated with seeds of variety DMRLSR-Y harvested 40 DAT and dried in the sun in year 2002. Seeds that were artificially dried were not infected by *Penicillium* sp., irrespective of variety and the stage of harvest. Table 4 also shows that seeds of cultivar DMRLSR-Y harvested 30 DAT and sun dried were associated with the highest percentage incidence (30.5%) of *Penicillium* spp.

DISCUSSION

This study was conducted with the main objective of determining the influence of the stage of harvest and drying methods on the germination of maize seeds and in the incidence of seed-borne fungi. Comparison was also made among the different drying methods. Seed metabolic activities generally increase with temperature and moisture content. Simultaneously, high moisture content reduced seed germination, and stimulates the life of microorganism. Therefore, storage has to be sufficiently safe by lowering the moisture content. This investigation shows that the stage of harvest significantly

influenced the germination of maize seed even after drying to the recommended moisture content. This implies that the higher the moisture content at harvest. the lower the percentage germination. This observation is similar to the reports of Harris and Lindblad (1978) who observed that the higher the moisture content the higher the loss of seed grain to pathogens. Among the recommendation for solving mycotoxin problems in cereals, rapid drying of agricultural products to low moisture is often emphasized, because all scenarios leading to mycotoxin contamination relate to nonmaintenance of stored products at safe moisture content. This study revealed that rapid drying of maize seeds artificially is better than shade and sun drying. Hamilton (2000) have also reported that drying harvested maize to 15.5% moisture content or lower within 24 to 48 h reduced the risk of fungi growth and consequently aflatoxin production. However, the adoption of the artificial drying method have been found to be low as it results in excessive consumption of fire wood and electricity, which may not be profitable for grains. Most African farmers spread their harvest to dry under the sun, which often require longer durations for the product to attain safe moisture and may be a difficult task due to the high rainfall at time of harvest.

Maize seeds from ears harvested 30 DAT and 35 DAT irrespective of the drying methods were uninfected with any of the field fungi observed in this study. This agrees with the reports of Amyot (1983) and Rachaputi et al. (2002) which advocated early harvesting as means of

^{*}Values are means of four replicates (100seeds/replicate/variety/year).

^{**} Means followed by the same letters in the same column are not significantly different

reducing infection by field fungi to minimize aflatoxin levels and obtain maximum returns. However, seeds from ears that were allowed to stay for more than 35 DAT were infected by F. moniliforme, F. graminearium, B. thebromae, and D. maydis. Penicillium spp. and Aspergillus spp. were isolated from the seeds irrespective of the stage of harvest and drying method. Zummo and Scott (1990), Owolade (1997) and Owolade et al. (2001, 2002) have previously observed that F. moniliforme, F. graminearium, B. thebromae, and D. maydis, Penicillium spp. and Aspergillus spp. are often encountered on maize seeds that were allowed to dry on the field before harvest. This work thus suggests that, for maize seeds to be free from major seed-borne field and store fungi, ears should be harvested at 35 DAT and dried either in the sun or under the shade or artificially. However, where sun drying is not possible due to high rainfall at harvest, maize ears can be harvested at 40 DAT and dried under the shade.

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