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Research Paper

Response of Maize Genotypes to *Fusarium Verticillioides* Strains from Two Agro Ecological Zones in Southwest Nigeria

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Abstract: *The reduction in the quantity and quality of maize production in Nigeria has been attributed to some seed borne pathogens, of which strains of Fusariumverticillioides causing ear rot infections are included. Hence, this study investigated the resistance of six maize genotypes to strains of F. verticillioides isolated from infected maize cobs obtained from rainforest (Ibadan) and derived savanna (Iloora) agro ecological zones of Southwest Nigeria. The screen house experiment was laid out in complete randomized design. The pathogenic strains of F. verticillioides isolated from five locations were inoculated at the silking stage (six weeks after planting) of maize cultivars. Data collected on the growth, yield and ear rot disease assessments were subjected to analysis of variance (ANOVA) using SAS 9.1 statistical software. AMA TZBR YCF was the most resistant genotype to the pathogens, followed by TZEI 25, while TZBR COMP 2-YCS 280, TZEI 22, (IBZA-EN13) TZBR COMP 2-YCS, and TZEI 161 were most susceptible. The decreasing order of genotypes in yield production were; AMA TZBR YCF, TZBR COMP 2-YCS 280, TZEI 25, TZEI 22, TZEI 161 and (IBZA-EN13) TZBR COMP 2-YCS. The F. verticillioides strains isolated from Ibadan (IBD 1, IBD 2, and IBD 3) were the most virulent in this study compared with Iloora (ILR 1 and ILR 2) which showed moderate virulence. Therefore, AMA TZBR YCF was the most resistant genotype to strains of F. verticillioides in this study, and could be most suitable for cultivation in locations where Fusarium ear rot diseases are endemic so as to ensure food security.*

Keywords: Maize genotypes, *F. verticillioides*, pathogenicity, food security.

Introduction

Maize (*Zea mays* L.) is one of the most economically important cereals in agro-ecological zone of Africa, especially in Nigeria (Olakojo*et al.*, 2001; IITA 2007; Olawuyi *et al*., 2010; Okoro-Robinson *et al*., 2014). Maize is susceptible to several pathogens that result to yield loss and decrease in the quality of production (Akande and Lamidi 2006). *Fusarium verticilliodes* is one of the most prevalent pathogens of maize causing root rot, stalk rot and ear rot in the tropical and subtropical regions of the world (Rossouw *et al*., 2002; Alankoya *et al*., 2008). Grain yield losses ranging from 0-70% are caused by some major diseases which depend on factors such as genetic constitution of the cultivars and stage of growth at the time of infections (Bua and Chelimo, 2010).

F. verticillioides causes the kernel from airborne conidia through silks and become symptomatic (Munkvold *et al*., 1997; Duncan, 2010). Moreover, another infection pathway is systematic through the seed as a result of inoculums that survive in crop through residue in soil (Alakonya 2008; Venturini *et al*., 2011).

Improvement of host plant resistance to this fungus provides the most feasible control options (Brown and Chen 1999). However, resistance to *Fusarium* ear rot is under genetic control and heritable resistance has been identified in maize.

Disease assessment constitutes one of the most suitable characters for investigating fungal pathogenic variability and can be quantified by several methods such as determination of disease incidence and severity (Zhan *et al*., 2007; Haque *et al*., 2008; Venturini *et al*., 2012). This study therefore aimed at investigating the variability in the response of maize genotypes to pathogenicity of *F. verticillioides* strains.

Materials and Methods

Sources of Host Plant and Pathogen

Six maize genotypes (TZBR COMP 2-YCS, TZBR COMP 2-YCS 280, AMA TZBR YCF, TZEI 22, TZEI 25 and TZEI 161) were collected from the genebank of the International Institute of Tropical Agriculture (IITA), Ibadan Nigeria, while *F. verticillioides* strains were isolated from diseased sample of maize collected from rainforest (Ibadan) and derived savanna (Ilora).

Preparation and Multiplication of Spore Suspension

The *F. verticillioides* were subcultured on Potato Dextrose Agar (PDA) to obtain the pure isolates. The multiplication of spore suspension were done and the solutions were then sieved with double folded cheese cloth to allow the passage of fungal spores which were counted separately for each fungus and re-adjusted to 1×10^6 spores/ml using haemocytometer. The mycelia growths of *F. verticillioides* strains were harvested separately in already sterilized beakers

Morphological and Microscopic Identification of *F. Verticillioides*

Isolation and identification of *F. verticillioides* were carried out according to Fusarium laboratory manual of identification described by Leslie and Summerell (2006).

Morphological identification of *F. verticillioides* isolates were based upon characters on cultured plates which include; rate of growth, pigment production, presence or absence of mycelium and colour of microconidia in sporodochial, while the microscopic identification of distinctive structures and characters of *F. verticillioides* were philiades, chlamydospores, microconidia and macroconidia.

The comparisons of these characters were done with the aid of the procedure of *Fusarium* laboratory identification manual described by Leslie and Summerell (2006).

Seed and Soil Sterilization

The top soil of 0-15cm deep was collected from the nursery farm of Botany Department, University of Ibadan, Nigeria. The soil was sterilized in an electric soil sterilizer. The maize seeds were treated separately with 5% NaOCl solution for 3 minutes, then rinsed in two exchanges of sterile distilled water and air dried in laminar flow for 2 hours.

Experimental Design

The pots were arranged in the screen house and factorially laid out in a completely randomized design. The six treatments consisted of; T1 = Control (Untreated maize), T2 = maize + FV strain 1, T3 = Maize + FV strain 2, T4 = Maize + FV strain 3, T5 = Maize + FV strain 4, T6 = Maize + FV strain 5, Where FV is the *F.verticillioides* strains. The maize genotypes were treated at the same time, while uninoculated maize served as control.

Planting and Pathogen Inoculation

Sandy-loam soil of 4 kg was filled up to three-quarter of the pot, and weighed into each of the 60 perforated pots spaced out 15 cm apart and labeled according to the treatments. Three viable seeds were planted per pot, and each pot was adequately maintained by regular watering, weeding and thinning to one stand after germination.

The maize ears were inoculated with strains of *F .verticillioides* spore suspension (1×10^6 spores/ ml) through the silk of the maize development according to the procedure of Cardwell *et al*. (1981).

Disease Assessment

The percentage incidence of ear rot symptoms were estimated as described by Michel *et al*., (1997).

$$\text{\% Disease incidence} = \frac{\text{No of plant showing ear rot symptom}}{\text{Total no of maize samples}} \times 100$$

Disease Severity Assessment

Scoring for disease severity in relation to the symptoms observed on growth and yield characters were done according to the method described by Kim (1994).

The ratings were;

1- Normal plant growth/ no visible damage symptom/ excellent yield
2- A slight infection / uniform, large and well- filled ears with excellent tassel and ear formation
3- Moderate infection/ good ears and tassels
4- Poor and stunted growth/ fair ears and tassels
5- Very poor and very heavy infection/ poor, rotten, small and no ear formation

Determination of Growth Agronomic and Yield Parameters

The following growth, agronomic and agronomic and yield parameter were taken according to the standard methods number of leaves, plant height (cm), stem height (cm), stem girth (cm), leaf area

(cm^2), ear aspect, ear harvest, ear rot, plant aspect, plant harvest and husk cover. The plant height (cm), stem height (cm), stem girth (cm) and leaf area (cm^2) were collected at 14 days intervals.

Data and Analysis

The data were subjected to analyses of variance (ANOVA) using SAS 9.1(2003) statistical software, while the means were separated at 95% confidence interval by Duncan's multiple range test (Duncan, 1955).

Results

The influence of genotype after inoculation of *F.verticillioides* produced highly significant effect ($p<0.01$) on all the growth characters of maize (Table 1). Also locations of maize pathogen had highly significant effect on the leaf area but significant for plant height, stem height and number of leaves. There was no significant effect of day after inoculation (DAI) on growth character of maize. The yield assessment also showed maize genotypes as producing highly significant ($p<0.01$) effect on the plant aspect, ear aspect, ear harvest, shoot weight, root weight and the husk cover that produced significant ($p<0.05$) results among the yield characters observed. Whereas, the result based on the location of different strains of the pathogens showed no significant effect on the yield except on husk cover that was significantly ($p<0.05$) affected. More so, no significant difference was observed in the replicated samples (Table 2).

The *F. verticillioides* treated maize genotypes in table 3 showed variations in their morphological response to the effect of the pathogen. G5 showed the most significant ($p<0.05$) on plant height (150.73cm) while TZEI 25 had the least (74.99cm). The stem girth of G4 is significantly higher but not different from G5 and TZEI 25, TZEI 161 and G1 had similar effect (Table 3). TZEI 25 produced the highest number of leaves (14.90) and leaf area (523.16cm). The strains of *F. verticillioides* isolated from two location in south western Nigeria caused significant ($p<0.05$) reduction in the growth of maize genotypes when compared to the untreated samples (control). The strain from D1(2)B IBD was observed as the most virulent in this study, followed by B2(2), then A1(3)a IBD, while those from D3 ILR and D5 ILR which exhibited similar level of virulence were not significantly ($p<0.05$) different from the control experiment in the plant height and stem girth performances. There was no significant effect on the location of pathogen, number of leaves and leaf area. Also, no significant difference was recorded at days 7 and 14 after inoculation of the pathogen (Table 3).

The result obtained in the yield assessment of maize genotype showed similar trends with that of growth characters. G5 produced the most significant ($p<0.05$) result for the shoot (6.59) and root weight (8.21), followed by G4 which was the most significant on the plant aspect (3.75), ear aspect (4.54) and husk cover (3.29), and then genotype TZEI 25, while TZEI 22, TZEI 161 and G1 produced relatively lowered level of significance. Furthermore, all the strains of *F. verticillioides* isolated from different location produced lower yield when compared to the untreated genotypes (control). There was no significance difference observed on the plant harvest, ear aspect, ear harvest, shoot weight and root weight on the strains from each location and the control experiment. However, the plant aspect and husk cover assessment showed B2 (2)IBD, D5 ILR, A1 (3) a IBD, D1 (2) B IBD and D3 ILR genotypes had the increasing effect of *F. verticillioides* on the yield characters respectively (Table 4).

Maize genotype TZEI 22 (64%) showed the highest level of disease severity followed by genotypes TZEI 25 (60.8%), TZEI 161 (57.6%), G5 (57.6%) and G4 (53.4%) while G1 (50.8%) had the least.Furthermore, the pathogen isolated from A1 (3) a IBD (70.8%) showed the most virulent, followed by D3 ILR (60.8%), D1(2)B IBD (59.2%), D5 ILR (58.4%), and B2(2) (58.4%). The pathogenic effect of *F. verticillioides* strain from different locations was established when compared with that of untreated maize genotypes (control) (Figure 1).

Table 1: Mean square effect of genotype and location on growth characters of maize after inoculationof *F. verticillioides*

Source	Df	Plant height	Stem girth	Number of leaves	Leaf area
Genotype	5	37209.69**	4.53**	353.54**	890562.64**
Locations of pathogen	5	3824.59*	2.13*	262.09*	980963.38**
DAI	1	0.0068ns	0.001ns	0.22ns	11.54ns
Replicates	3	8214.65ns	6.28ns	246.28ns	655900.60ns
Error	273	373387	195	19693.6	64119460
Corrected Total	287	602150	247.21		28936

** Highly Significant at P< 0.01
* Significant at P< 0.05

Table 2: Mean square effect of genotype and location on yield characters of maize after inoculation of *F. verticillioides*

Source	Df	Plant aspect	Plant harvest	Ear Aspect	Ear harvest	Husk cover	Shoot weight	Root weight	Ear rot
Genotype	5	4.58**	0.31ns	1.90**	3.28**	6.51*	41.89**	48.99**	1.42ns
Location of pathogen	5	1.96ns	0.32ns	0.67ns	0.31ns	6.71*	9.65ns	6.84ns	7.52**
Replicate	3	2.27ns	0.19ns	0.17ns	1.34ns	2.03ns	32.68ns	16.69ns	0.21ns
Error	130	96.47	20.6	61.67	70	357.35	792.59	1879.08	293.21
Corrected Total	143	135.97	24.33	75	91.97	429.49	1109.75	2208.25	338.49

** Highly Significant at P< 0.01
* Significant at P< 0.05

Table 3: Morphological response of maize genotypes to *F. verticillioides*

Parameters	**Variables**	**Plant height (cm)**	**Stem girth (cm)**	**Number of leaves**	**Leaf area (cm^2)**
Genotype	G4	124.91b	2.73a	11.73abc	219.67b
	G5	150.73a	2.67a	12.06ab	247.94b
	TZEI 25	74.99e	2.05b	14.90a	523.16a
	TZEI 22	108.42c	2.58a	10.83bcd	206.00b
	TZEI 161	85.64de	2.17b	7.50d	183.68b
	G1	94.28cd	2.05b	8.21cd	145.12b
Locations of pathogen	Control	118.30a	2.70a	15.36a	540.30a
	D3 ILR	113.70a	2.50a	10.65b	230.30b
	A1(3)a IBD	113.00a	2.41ab	10.27b	186.30b
	D5 ILR	114.29a	2.55a	10.53b	203.30b
	D1(2)B IBD	95.11b	2.05b	8.69b	188.70b
	B2(2)IBD	108.10ab	2.59a	10.27b	182.10b
DAI	7	106.49a	2.38a	10.90a	254.06a
	14	106.50a	2.39a	10.84a	254.46a
Replicate	1	91.83b	1.97b	8.89b	171.00b
	2	106.75a	2.42a	9.81ab	178.97b
	3	115.80a	2.70a	12.56a	317.00ab
	4	111.59a	2.48a	12.24a	350.08a

DAI- Days after inoculation
Means with the same alphabets are not significantly different from each other while means with different alphabets are significantly different from each other.

Table 4: Agronomic and yield response of maize genotypes to *F. verticillioides*

Parameters	**Variables**	**Plant aspect**	**Plant harvest**	**Ear Aspect**	**Ear harvest**	**Husk cover**	**Shoot weight (g)**	**Root weight (g)**
Genotypes	G4	3.75a	0.67b	4.54a	0.92c	3.29a	4.07c	7.18ab
	G5	3.29ab	0.71ab	3.79c	1.50ab	1.79b	6.59a	8.21a
	TZEI 25	3.08b	0.88ab	4.42ab	1.08bc	2.75ab	5.69ab	7.07ab
	TZEI 22	2.5c	0.96a	4.08bc	1.21bc	2.71ab	4.54bc	6.67ab
	TZEI 161	3.54ab	0.79ab	4.21ab	1.83a	2.79ab	3.18c	4.21c

	G1	3.42ab	0.71ab	4.46ab	0.88c	3.13a	3.49c	5.41bc
Location of pathogen	Control	3.67a	0.90a	4.33a	1.17a	3.54a	4.21a	5.75a
	D3 ILR	2.83c	0.83a	4.08a	1.42a	2.79ab	4.64a	6.31a
	A1(3)aIBD	3.33abc	0.83a	4.50a	1.21a	2.38b	4.75a	7.28a
	D5 ILR	3.25abc	0.58b	4.33a	1.13a	3.13ab	4.64a	6.29a
	D1(2)BIBD	3.08bc	0.88a	4.08a	1.33a	2.08b	4.99a	6.86a
	B2(2)IBD	3.42ab	0.88a	4.12a	1.17a	2.54ab	4.33a	6.26a
Replicate	1	3.39ab	0.72a	4.28a	1.25ab	2.42a	4.32b	5.45a
	2	3.08b	0.86a	4.33a	1.47a	2.72a	4.71ab	6.96a
	3	3.03b	0.83a	4.19a	1.22ab	2.89a	5.81a	6.76a
	4	3.56a	0.72a	4.19a	1.00b	2.94a	3.52b	6.65a

Means with the same alphabets are not significantly different from each other while means with different alphabets are significantly different from each other.

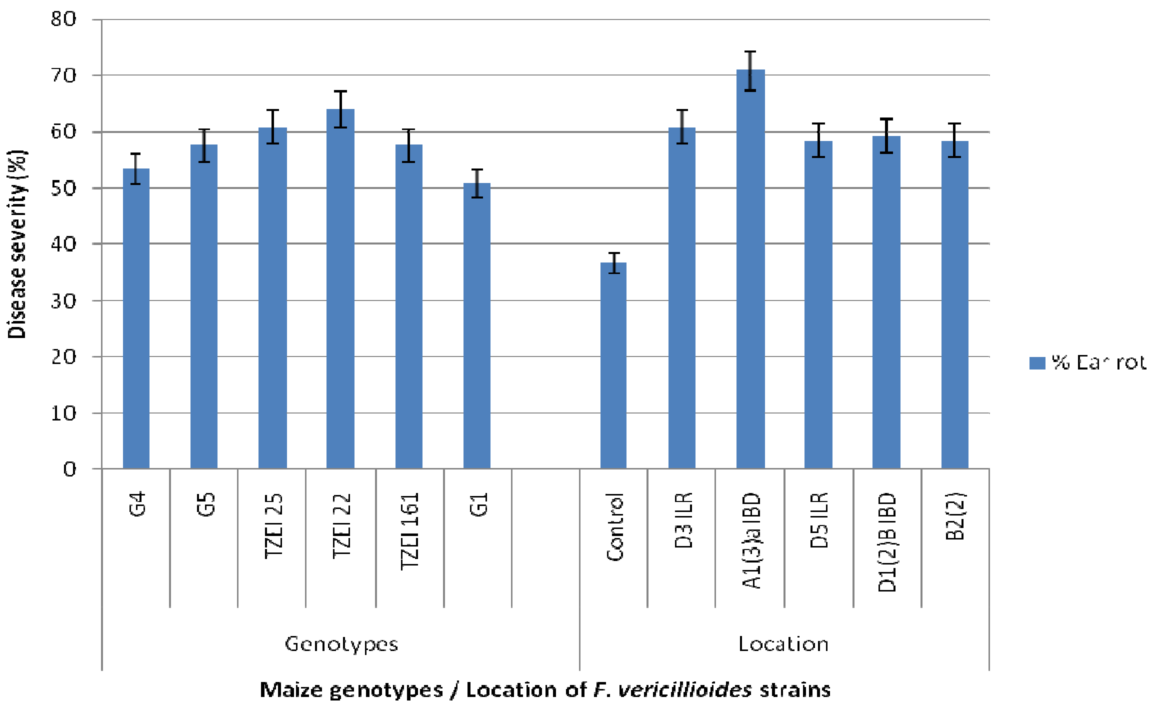

Figure 1: The percentage disease severity in maize genotypes by *F. verticillioides* strains from different locations

Discussion

The findings from this study shows that, there was variability in pathogens isolated from different maize hosts from diverse locations as similarly observed by Zhan *et al*. (2007) and Haque*et al*. (2008). The response of maize genotypes TZBR COMP 2-YCS, TZBR COMP 2-YCS 280, AMA

TZBR YCF, TZEI 22, and TZEI 161 to different strains of *F. verticilliodes* isolated from maize obtained from two Agroecologocal Zones were investigated in this study.

The effect of *F. verticillioides* strains on the morphological traits of the maize genotypes evaluated in this study was in accordance with Machungo*et al*. (2009), who reported that plant responses to endophyte treatments was assessed on plant height, girth, number of functional leaves, length and width of the youngest leaves and root and shoot weights. The maize genotypes showed variation in the morphological responses to the effect of *F. verticillioides*. AMA TZBR YCF showed the most significant growth of the plant height and stem girth, followed by TZEI 25 which had the best supported number of leaves and leaf characters, which agrees with the report of Khalil *et al*.(2003).However, reduction in growth of maize genotypes was observed, this is in agreement with the report of Munkvold*et al.,(*1997) who reported that *Fusarium*ear rot negatively affects the physical, physiological and phytosanitary qualities of maize seed, as supported by the findings of Vigier*et al.,* (1997). The strain D1(2)B from Ibadan was found to be the most virulent in this study, which is in-line with the observation made byAkanmu*et al*., (2013), while strains from Iloora D3 and D5 which were found to exhibit similar level of virulence but rated to be the least, this means that despite being morphologically different, some strains of *F. verticillioides* might be genetically identical.

The maize genotypes as well showed variation in their yield responses to the effect of *F. verticillioides*. AMA TZBR YCF also showed the most significant result for the shoot and root weight, as well as TZBR COMP 2-YCS 280 which had the best significant result on the plant aspect, ear aspect and husk cover is in agreement with the findings of Elisabeth *et al*., (2008). This variation may be due to stimulation of growth and development of maize plants caused by *F. verticillioides* infection as a result of production of plant growth-promoting hormones (Yates *et al*., 1995). On the other hand, the reduction in yield characters of maize genotypes as a result of *F. verticillioides* infection validated the reports of Saremi *et al.* (2011) who reported that the negative effect of *Fusarium* species on crops infection and yield reductions could be attributed to the secretion of mycotoxins. Reduction in yield were observed on the plant harvest, ear aspect, ear harvest, shoot weight and root weight by the strains from each location. Conversely, the suppressive effect of *F. verticillioides* strains B2(2), D5 ILR, A1(3)a IBD, D1 (2)B IBD and D3 ILR on the plant aspect and husk cover of the maize genotypes could be attributed to secretion of mycotoxin and in accordance with the observation made by van der Burgt (2009).

There was of genetic variability of the maize genotype to disease severity caused by *F. verticillioides*. This implies that the maize genotypes responded differently to *F. verticillioides,* and this also revealed the presence or absence of host resistance genes in different maize genotypes as reported by Naidoo*et al*. (2002), Hefny *et al*. (2012)and Fasihi *et al*. (2013) who confirmed the genetic resistance of maize to ear rot disease caused by *F. verticillioides*strains. There were also differences in the rate of symptom development on maize genotypes, suggesting that there were variations in virulence level of the *F. verticillioides* strains isolated from different locations. Disease symptoms of *F. verticillioides* vary widely and range from asymptomatic infection to severe rotting of all plant parts (Oren, 2003). This may be due to environmental conditions, water availability and the genetic background of the plant and the pathogen which may all be important factors in disease development (Dodd, 1980; Kedera *et al*., 1994).

Conclusion and Recommendation

AMA TZBR YCF found to be the most resistant genotype to *F. verticillioides*, it could therefore be recommended for cultivation in locations where *Fusarium* ear rot diseases are endemic. This genotype should also be considered for selection and hybridization in future breeding programmes.

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