



Resistance of Kenyan wheat germplasm to *Fusarium* head blight and deoxynivalenol contamination

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Article published on August 14, 2016

Key words: Cultivar resistance, Deoxynivalenol, *Fusarium* head blight, Wheat.

Abstract

Fusarium head blight (FHB) of wheat causes quantitative and qualitative reduction in yield. Cultivar resistance is the most effective method of managing the disease. This study evaluated the resistance of wheat germplasm currently available in Kenya to *Fusarium* head blight and deoxynivalenol (DON) contamination. Nine wheat varieties and four CIMMYT lines were evaluated for susceptibility to FHB under two diverse agro-ecologies in Nakuru and Narok Counties, Kenya during the 2013 cropping season and in the greenhouse. The varieties and lines were inoculated at mid-anthesis with mixed inocula of three isolates of *F. graminearum*. Incidence and severity of FHB were assessed weekly and data on severity used to calculate the area under disease progress curve (AUDPC). After harvest, incidence of *F. graminearum* in the grain was determined and DON contamination determined by direct competitive enzyme-linked immunosorbent assay (ELISA). Incidence and severity of FHB differed significantly ($p \leq 0.05$) among the varieties and lines with variety Kwale showing the least disease while line 10155 had the highest FHB levels. The AUDPC ranged from 69.8 to 120.1 for the least and most susceptible varieties, respectively. All the wheat lines and varieties accumulated DON ranging from 442 to 748 ng/g (Mean = 572 ng/g). There was a positive correlation between FHB severity, AUDPC, re-isolation frequency of *F. graminearum* and DON accumulation. The assessed wheat varieties and lines could be grouped into two categories: moderately tolerant and susceptible. Wheat varieties and lines available in Kenya are susceptible to FHB and DON contamination implying need for considering other strategies for managing FHB.

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Introduction

Fusarium head blight (FHB), which is caused by multiple *Fusarium* species is one of the most important diseases of wheat (*Triticum aestivum*) worldwide. *Fusarium graminearum* Schw. [teleomorph: *Gibberella zeae* Schw. (Petch.)] predominates in most parts of the world and has been reported as the most important species causing major FHB epidemics in the last two decades (Kazan *et al.*, 2012). *Fusarium culmorum*, *F. poae*, *F. sporotrichoides*, *F. avenaceum* and *Microdochium nivale* also play a significant role in causing the disease (Mesterhazy *et al.*, 2005). Geographical distribution and predominance of a particular *Fusarium* species is related to temperature requirements of the species (Parry *et al.*, 1995).

The aggressiveness of *F. graminearum* involves different mechanisms such as production of various enzymes that degrade cell wall, which are important in colonization and establishment of *Fusarium* head blight (Ortega *et al.*, 2013). *Fusarium* head blight causes severe yield losses of up to 70% and also affects the quality of grains by reducing protein content and inducing color defects. *Fusarium graminearum* also produces various mycotoxins such as deoxynivalenol and its acetylated derivatives such as 15 Acetyl-DON and 3 Acetyl-DON, which are important for full virulence on wheat ears (Desjardin, 2006; Stepien *et al.*, 2010). Low or lack of production of these mycotoxins during infection results in enhanced plant defense mechanism in form of cell wall thickening which is known to impede rachis colonization. Contamination of wheat grains with mycotoxins especially DON causes vomiting, diarrhea, fever and other symptoms in humans and animals (Pestka *et al.*, 2004; Cirlini *et al.*, 2014).

Appropriate methods of land preparation, rotation programs with non-cereal crops, good crop husbandry, seed treatment, timely harvesting and proper storage may help reduce the level of FHB inocula (Parry *et al.*, 1995). However, these methods alone cannot effectively manage FHB due the large amount of inocula in the soil and crop residues (Gilbert and Tekauz, 2011).

Therefore, the most effective approach is to integrate multiple strategies (McMullen *et al.*, 2008) like breeding for host resistance, application of effective fungicides, residue management and disease forecasting (Wegulo *et al.*, 2011). Use of FHB resistant varieties is the most viable control strategy for FHB and associated mycotoxins (D' Mello *et al.*, 1999; Oliver *et al.*, 2005). However, there are no resistant wheat cultivars that are documented (Mesterhazy *et al.*, 2011). Types of resistance to FHB include type I (resistance to initial infection of spikelets), type II (resistance to spread of the pathogen within spikes (Schroeder and Christensen, 1963) and resistance to DON accumulation (Mesterhazy, 1995). The last two have not been exploited since their mechanisms are not well understood (Zhang *et al.*, 2011). Chinese Sumai 3 spring wheat line and its derivatives like Saikai 165 are known sources of type II resistance (Kubo *et al.*, 2013). A number of traits such as plant height, presence of awns, spike compactness and heading date have been associated with FHB resistance (Chrпова *et al.*, 2010). The objective of this study was therefore to evaluate wheat varieties and lines available in Kenya for resistance to *Fusarium* head blight and accumulation of deoxynivalenol.

Materials and methods

Description of experimental materials and trial sites

Nine wheat varieties obtained from Kenya Agricultural and Livestock Research Organization (KALRO) and four International Maize and Wheat Improvement Centre (CIMMYT) lines were evaluated for resistance to *Fusarium* head blight and DON accumulation. The varieties were Kenya Sunbird, Kwale, Robin, Korongo, Njoro BW2, Kenya Wren, Kibis, Chiriku, Kenya Hawk while the CIMMYT lines were 957, 4969, 10155 and 10213. Evaluation of resistance of wheat varieties and lines to FHB was conducted at two field sites and in the greenhouse. The field trials were conducted in two agro-ecological zones: upper midland four (UM4) in Nakuru County (Soo.28088, E036.03308) at an altitude of 1885m ASL, and

upper highland two (UH2) in Narok County (Soo.78766, E035.89093) at an altitude of 2560m ASL. Nakuru and Narok Counties are the major wheat growing regions in Kenya and are characterized by good soils, adequate rainfall and plenty of land. Two greenhouse trials were conducted at the University of Nairobi's Field Station. Trials at the three sites were conducted from June to September 2013. The mean annual rainfall, minimum and maximum temperature in Nakuru County during the experimental period were: 149.4mm, 12.7°C and 24.5°C, respectively while the corresponding conditions in Narok County were 27.3mm, 9.6°C and 23.4°C.

Growth of wheat plants

In the field, each of the nine varieties and four lines was drilled in lines spaced at 20 cm in 1m² plots and replicated three times, with 1M paths between the plots. The experiment was arranged in randomized complete block design (RCBD) (Gomez and Gomez, 1983). In the greenhouse, twenty seeds of each variety and line were planted in each 20cm diameter pot containing a mixture of soil and farm yard manure (2:1 v/v) and replicated four times and arranged in completely randomized design (Gomez and Gomez, 1983). Flowering dates of various lines and varieties were synchronized by early planting of late maturing varieties and late planting of early maturing varieties to ensure they flowered at the same time. For greenhouse trials, the plants were allowed to grow outside the greenhouse until flowering stage (GS 60). Standard agronomic practices, excluding fungicide application were carried out. The plants were fertilized with di-ammonium phosphate (DAP, 18:46:0) at planting, NPK (24:24:18) at tillering stage (GS 13) (Zadoks *et al.*, 1974) and foliar fertilizer Bayfolan (NPK 24:24:18) at stem elongation stage (GS 30-31) at the rate of 1.5L per Ha. The insecticide Karate® (Lambda-cyhalothrin) was applied at GS 12 at the rate of 150 mL/ha to protect the plants from infestation by aphids and leaf chewing insects while weeds were controlled manually as required.

Preparation of inoculum and inoculation of wheat plants

A mixed inoculum of three isolates of *F. graminearum* which were originally isolated from wheat and soil samples from Narok County in Kenya was used in all the experiments. Each isolate was cultured separately on potato dextrose agar (PDA) and cultivated on mung bean medium (Bai and Shanner, 1994) to produce enough macroconidia. The mung bean medium was prepared by boiling 40g of mung bean in 1000 mL of water for 15 minutes. The extract was filtered through cheese cloth and 100 mL portions of the extract were autoclaved at 121°C for 20 minutes at 15psi. Each flask containing the cooled mung bean extract was inoculated with two agar discs cut from 5 day-old cultures of the *F. graminearum* isolate grown on PDA. The cultures were incubated in a New Brunswick Scientific C25 shaker (Artisan Technology Group, Illinois, USA) at 100 strokes per minute for four days followed by a further seven days under stationary conditions at 25°C. Spore suspension for each isolate was blended, filtered through cheese cloth, spore concentration determined and adjusted to 1×10⁵ spores/mL using a haemocytometer. The resultant inoculum from each isolate was mixed in equal proportions (v/v).

Inoculation of each variety/line was done early in the morning and late in the afternoon at mid-anthesis (50% flowering) by uniformly spraying approximately 20mL of the inoculum to the ears with the spore suspension using a hand sprayer and ensuring that all the spikes were exposed to the inoculum. Control plots were sprayed with sterile water. The inoculated ears were covered with a polythene bags for 48 hours to ensure high relative humidity for optimal infection (Muthomi *et al.*, 2008).

Assessment of Fusarium head blight and determination of grain weight

Wheat ears were visually examined for *Fusarium* head blight symptoms on spikelets seven days after inoculation. Severity of *Fusarium* head blight was

determined as the proportion of bleached spikelets on a scale of 0 – 9 (Miedaner *et al.*, 1996: 1 = no symptoms, 2 = <5%, 3 = 5-15%, 4 = 16-25%, 5 = 25-44%, 6 = 46-65%, 7 = 66-85%, 8 = 86-95%, 9 = 96-100%). Ten average sized ears per plot and per pot in the greenhouse were tagged and assessed weekly for four weeks for FHB development. Area under disease progress curve was calculated from FHB severity data using the formula by Shanner and Finney (1977):

$$\text{AUDPC} = \sum_{i=1}^n [(y_i + 1 + y_{i+1})][t_{i+1} - t_i]$$

where: y_i is visual score of FHB symptoms at the i^{th} observation; n - total number of observation days at the i^{th} observation; t - time. The area under disease progress curve was used as a measure for resistance, where the greater the value of AUDPC, the more susceptible the variety or line (Grausgruber *et al.*, 1995). At maturity (GS 92), ears in each plot or pot were harvested separately and grain weight of twenty random ears determined.

The ears were threshed manually by hand and the harvested grains were subdivided in two subsamples, one for re-isolation of *F. graminearum* and the other for DON analysis.

Re-isolation of Fusarium graminearum

The harvested kernels from every plot or pot were thoroughly mixed and surface sterilized in 1.3% sodium hypochlorite (NaOCl) followed by rinsing in three changes of sterile distilled water.

The kernels were blot dried in the lamina flow and five kernels aseptically plated on low strength PDA amended with mineral salts (PDA 17g, 0.5g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5g KCl, 1g KH_2PO_4 , 1.0g KNO_3) and antimicrobial agents (50mg tetracycline, 50mg streptomycin) (Muthomi, 2001) and replicated three times. After five days, *Fusarium* spp. were sub-cultured in PDA and synthetic nutrient agar, SNA (1.0g KH_2PO_4 , 1.0g KNO_3 , 0.5g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5g KCl, 0.2g glucose, 0.2g sucrose, and 20g agar (Nirenberg, 1981). Cultures on SNA were incubated under UV light for 14 days to facilitate sporulation while those on PDA were incubated at 25°C for 10 days.

Fusarium graminearum isolates were identified to species level based on cultural and morphological characteristics as described by Nelson *et al.* (1983) and Leslie and Summerell (2006). Re-isolation of *F. graminearum* was determined by counting kernels showing *F. graminearum* growth and used to calculate the percentage of infected kernels.

Determination of deoxynivalenol level in wheat grains

Grain samples harvested from the inoculated ears were analyzed for deoxynivalenol content by competitive enzyme-linked immunosorbent assay (ELISA). The grain samples were mixed and ground using the Romer analytical sampling mill (RAS®, Romer Labs, Missouri, USA) and stored at 4°C. Deoxynivalenol was extracted from five grams of the homogenized, ground sample with 25 mL of distilled water. The sample was then shaken vigorously at 300 revolutions per minute in a mechanical shaker (Vortex mixer, Bibby Scientific Limited, United Kingdom). The extract was centrifuged for 10 minutes at 3000 rpm in ultra speed centrifuge (Rasayanika, India). The ELISA procedure was carried out following manufacturer's instructions using Ridascreen® Fast DON Kit (Biopharm GmbH, Darmstadt, Germany). Correlation analysis was done to determine the relationship among the various disease, infection and DON contamination parameters.

Data analysis

Data were subjected to analysis of variance (ANOVA) using the PROC ANOVA procedure of Genstat (Lawes Agricultural Trust, Rothamsted Experimental Station 2006, version 9) and differences among the treatment means compared using Fisher's protected LSD test at 5% probability level. The correlation between various parameters expressed by FHB index was computed by spearman's correlation (Payne *et al.*, 2008).

Results

Resistance of wheat lines and varieties to FHB and DON contamination

All the wheat lines and varieties tested were susceptible to *F. graminearum* although there was variability in the levels of susceptibility both in the field and in the greenhouse (Table 1). Overall, the disease was higher in Narok than in Nakuru County. There were significant ($P \leq 0.05$) differences in severity of FHB among the lines and varieties inoculated with *F. graminearum*. The mean disease severity ranged from 30.0% for variety Kwale to 54.4% for variety Kenya Sunbird in the field experiment.

The area under disease progress curve ranged from 69.8 for the least susceptible variety to 116.3 for most susceptible variety in the field experiment. In the greenhouse trial, mean severity ranged from 31.1% for variety Kwale to 56.7% for line 10155. The area under disease progress curve ranged from 69.8 for the least susceptible variety to 116.3 for most susceptible variety in the field experiment and 70.8 to 120.1 for the greenhouse trials (Table 6). There were significant ($P \leq 0.05$) differences in severity of FHB among the lines and varieties inoculated with *F. graminearum*.

Table 1. Mean incidence, severity and AUDPC of *Fusarium* head blight on wheat ears of different varieties and lines inoculated with *F. graminearum* in field trials conducted in Nakuru and Narok Counties.

Variety/Line	Incidence (%)		Severity (%)		AUDPC	
	Nakuru	Narok	Nakuru	Narok	Nakuru	Narok
Sunbird	20.4	17.1	46.7	63.0	98.3	134.4
Kwale	6.9	5.3	24.4	36.7	54.9	84.8
Robin	8.3	10.2	23.3	54.4	55.7	116.3
Njoro BW 2	17.9	7.2	30.0	44.4	64.3	96.4
Korongo	7.6	9.2	31.1	53.3	67.5	115.5
Kenya Hawk	7.6	14.8	23.3	51.1	50.4	108.5
Kenya Wren	14.2	15.7	38.9	54.4	80.5	116.8
Kibis	6.7	7.5	26.7	47.8	58.1	101.9
Chiriku	8.4	10.2	27.8	50.0	55.1	109.3
957	27.0	36.1	35.6	48.9	76.4	105.0
4969	20.1	54.4	34.4	60.0	74.3	127.8
10155	16.5	40.0	38.9	67.7	83.0	144.0
10213	25.1	34.7	33.3	52.2	72.7	110.4
LSD ($p \leq 0.05$)	16.2	10.7	10.0	4.4	19.6	11.9
CV (%)	13.6	18.9	4.9	0.8	4.0	1.7

Means followed by the same letter(s) within each column are not significantly different at $P \leq 0.05$; LSD: least significant difference; CV: coefficient of variation, AUDPC: Area under disease progress curve.

Fusarium graminearum was re-isolated from all the lines and varieties although there were significant differences ($p \leq 0.05$) in re-isolation frequency (Table 2). The pathogen was re-isolated in high incidence from varieties Kenya Sunbird, Korongo, and lines 10155, 10213, and 4969 while it was least re-isolated from varieties Njoro BW2, Wren, Kwale and Kibis.

The pathogen was re-isolated in higher frequency in lines and varieties planted in Narok County compared to the same lines and varieties planted in Nakuru County. In the greenhouse trials, *F. graminearum* was re-isolated in high incidence from lines 10155, 4969, 957, and varieties Kibis and Kenya sunbird. The fungus was re-isolated in low incidence in varieties Kenya Hawk, Kwale, Njoro BW2 and Robin.

All the lines and varieties tested were contaminated with DON at concentrations ranging from 457 to 748 ng/g for the field trial and 442 to 720 ng/g for the greenhouse trial (Table 3).

Wheat kernels of variety Chiriku and line 10155 in the field trials had the lowest and highest DON levels, respectively while in the greenhouse trials, variety Kibis and line 4969 had the lowest and highest DON contamination, respectively.

Table 2. Re-isolation frequency (%) of *Fusarium graminearum* from wheat varieties and lines from field and greenhouse experiments.

Wheat variety	Field trials					Greenhouse trials				
	Nakuru		Narok		Mean	Cycle 1		Cycle 2		
Sunbird	51.1	ab	40.0	bc	45.6	abcd	57.8	b	53.3	bcd
Kwale	24.4	cd	46.7	abc	35.6	bed	28.9	cd	26.7	d
Robin	24.4	cd	48.8	abc	36.7	bed	35.6	bed	35.6	cd
Njoro BW 2	22.2	d	37.8	bc	30.0	d	35.6	bed	37.8	cd
Korongu	35.6	bcd	62.2	ab	48.9	abc	35.6	bed	55.6	bc
Kenya Hawk	33.3	bcd	53.3	ab	43.3	abcd	22.2	d	46.7	bcd
Kenya Wren	22.2	d	42.2	bc	32.2	cd	26.7	cd	51.1	bcd
Kibis	42.2	abcd	31.1	c	36.7	bed	51.1	bc	44.4	bcd
Chiriku	24.4	cd	53.3	abc	38.9	abcd	42.2	bed	57.8	bc
957	46.7	abcd	42.2	bc	44.4	abcd	42.2	bed	62.2	bc
4969	40.0	abcd	55.6	abc	47.8	abcd	55.6	bed	68.9	ab
10155	40.0	abcd	71.1	a	55.6	a	84.4	a	91.1	a
10213	60.0	a	42.2	bc	51.1	ab	35.6	bed	40.0	cd
LSD ($p \leq 0.05$)	19.4		22.3		15.1		21.6		23.7	
CV (%)	11.2		14.8		3.7		10.3		11.5	

Means followed by the same letter(s) within each column are not significantly different at $P \leq 0.05$; LSD: least significant difference; CV: coefficient of variation.

Table 2. Re-isolation frequency (%) of *Fusarium graminearum* from wheat varieties and lines from field and greenhouse experiments.

Wheat variety	Field trials					Greenhouse trials				
	Nakuru		Narok		Mean	Cycle 1		Cycle 2		
Sunbird	51.1	ab	40.0	bc	45.6	abcd	57.8	b	53.3	bcd
Kwale	24.4	cd	46.7	abc	35.6	bed	28.9	cd	26.7	d
Robin	24.4	cd	48.8	abc	36.7	bed	35.6	bed	35.6	cd
Njoro BW 2	22.2	d	37.8	bc	30.0	d	35.6	bed	37.8	cd
Korongu	35.6	bcd	62.2	ab	48.9	abc	35.6	bed	55.6	bc
Kenya Hawk	33.3	bcd	53.3	ab	43.3	abcd	22.2	d	46.7	bcd
Kenya Wren	22.2	d	42.2	bc	32.2	cd	26.7	cd	51.1	bcd
Kibis	42.2	abcd	31.1	c	36.7	bed	51.1	bc	44.4	bcd
Chiriku	24.4	cd	53.3	abc	38.9	abcd	42.2	bed	57.8	bc
957	46.7	abcd	42.2	bc	44.4	abcd	42.2	bed	62.2	bc
4969	40.0	abcd	55.6	abc	47.8	abcd	55.6	bed	68.9	ab
10155	40.0	abcd	71.1	a	55.6	a	84.4	a	91.1	a
10213	60.0	a	42.2	bc	51.1	ab	35.6	bed	40.0	cd
LSD ($p \leq 0.05$)	19.4		22.3		15.1		21.6		23.7	
CV (%)	11.2		14.8		3.7		10.3		11.5	

Means followed by the same letter(s) within each column are not significantly different at $P \leq 0.05$; LSD: least significant difference; CV: coefficient of variation.

Correlation among FHB, kernel infection and deoxynivalenol content

In the field trials, DON content was positively correlated ($p \leq 0.05$) to FHB incidence and severity, AUDPC and re-isolation of *F. graminearum* (Table 4). The AUDPC was also positively correlated to FHB incidence and

disease severity while the re-isolation frequency of *F. graminearum* was positively correlated to FHB incidence, severity and AUDPC. In the greenhouse trial, the AUDPC was strongly positively correlated to FHB severity ($r = 0.99, p = 0.05$) (Table 5), while the re-isolation frequency of *F. graminearum* positively correlated ($p \leq 0.05$) to FHB severity and AUDPC.

Table 3. Deoxynivalenol content (ng/g) in wheat kernels harvested from field and greenhouse trials.

Variety/Line	Field			Greenhouse		
	Nakuru	Narok	Mean	Cycle 1	Cycle 2	Mean
Sunbird	648.8	758.6	703.7	707.3	615.5	661.4
Kwale	626.9	776.2	701.6	760.9	189.5	475.2
Robin	601.8	763.2	682.5	721.1	538.3	629.7
Njoro BW 2	412.8	705.0	558.9	678.2	317.9	498.1
Korongo	587.9	752.4	670.2	592.6	531.4	562.0
Kenya Hawk	536.7	790.7	663.7	782.3	565.8	674.1
Kenya Wren	220.1	710.4	465.3	468.7	602.5	535.6
Kibis	282.0	710.4	496.2	686.7	198.7	442.7
Chiriku	166.0	747.9	457.0	705.0	260.6	482.8
957	534.4	766.2	650.3	623.2	577.3	600.3
4969	695.7	731.0	713.4	763.2	676.7	720.0
10155	725.7	770.8	748.3	522.2	613.2	567.7
10213	698.1	756.8	727.5	664.5	518.4	591.5
Mean	518.2	749.2	633.7	667.4	477.4	572.4

Ranking of resistance of wheat germplasm to FHB and DON contamination

The most susceptible wheat varieties to FHB were Kenya Sunbird and lines 10155, 957, 4969, 10213 while Kwale, Robin, Njoro BW2, Korongo, Kenya Hawk, Kenya Wren, Kibis, and Chiriku were rated as moderately resistant varieties (Table 6).

Based on disease severity, area under disease progress curve and deoxynivalenol content in the kernels, the wheat lines and varieties could be grouped in to two broad categories: moderately tolerant (Kwale, Robin, Njoro BW2, Korongo, Kenya Hawk, Kenya Wren, Kibis, and Chiriku) and susceptible (Kenya Sunbird and lines 10155, 957, 4969, 10213).

Table 4. Correlation coefficients among FHB incidence, severity, kernel infection, grain yield and deoxynivalenol content calculated from the field trials.

	Incidence	Severity	AUDPC	Re-isolation	DON
Incidence	-				
Severity	0.50*	-			
AUDPC	0.47*	0.99**	-		
Re-isolation	0.47*	0.99**	1.00**	-	
DON	0.25*	0.16*	0.18*	0.18*	-

* Significant correlation, ** highly significant correlation, ns – not significant at $p \leq 0.05$. DON - Deoxynivalenol, AUDPC - area under disease progress curve.

Discussion

All the wheat lines and varieties assessed showed FHB symptoms following inoculation with *F. graminearum* but they differed significantly in FHB severity and AUDPC. Cultivar Kenya Sunbird, and lines 10155, 10213, 957, 4969 were the most susceptible whereas Kwale and Njoro BW2 showed low levels of FHB in the field and greenhouse trials.

These results concur with findings by Muthomi *et al.* (2007) who reported that wheat varieties grown in Kenya were susceptible to FHB. Susceptibility of the varieties and lines to FHB implied that they do not possess genes for resistance to infection by *Fusarium* spp. When infected early, susceptible varieties produce *Fusarium* damaged kernels that are usually contaminated with mycotoxins.

Table 5. Correlation coefficients among FHB severity, kernel infection, grain yield and deoxynivalenol content calculated from the greenhouse trials.

	Severity	AUDPC	Re-isolation	DON
Severity	-			
AUDPC	0.99**	-		
Re-isolation	0.65**	0.65**	-	
DON	0.28*	0.30*	0.04 ^{ns}	-

* Significant correlation, ** highly significant correlation, ns – not significant at $p \leq 0.05$. DON - Deoxynivalenol, AUDPC - area under disease progress curve.

Variety Kwale which showed the lowest level of FHB has been previously rated as moderately susceptible while Njoro BW 2 was reported as moderately resistant (Muthomi *et al.*, 2002). The low levels of FHB on Kwale and Njoro BW 2 could be associated with inherent genetic resistant factors (Muthomi *et al.*, 2007), and genotype-environmental interaction or disease escape (Harnandes-Nopsa, 2010). Upon infection, the variations in gene expression patterns between FHB resistance and susceptible lines and varieties could be due to their genetic differences (Jia *et al.*, 2009). In addition, taller wheat varieties tend to show less FHB in the field, which has been attributed to the long distance between the wheat ear

and the soil which is the source of inoculum (Ramson and McMullen, 2008). Higher level of FHB was observed in the trial conducted in Narok County compared to trials in Nakuru County and the greenhouse. The difference in the level of disease in the two field trial sites could be attributed to variations in weather conditions. Based on the findings of this study, the evaluated lines and varieties can be classified as moderately tolerant and susceptible. Varieties Kwale, Kibis, Robin, Njoro BW2, Korongo, Hawk, Wren and Chiriku can be categorized as moderately tolerant while variety Kenya Sunbird and lines 4969, 957, 10155 and 10213 are susceptible.

Table 6. Disease severity, area under disease progress curve (AUDPC), re-isolation frequency, deoxynivalenol content and overall rating of the varieties and lines inoculated with three isolates of *Fusarium graminearum*.

Variety/Line	FHB Severity (%)		AUDPC		Re-isolation (%)		DON content (ng/g)		Overall rating
	Field	Greenhouse	Field	Greenhouse	Field	Greenhouse	Field	Greenhouse	
Sunbird	54.4 a	52.0 b	116.3 a	112.5 ab	45.6	55.6	703.7	661.4	S
Kwale	30.0 d	31.1 h	69.8 d	70.8 f	35.6	27.8	701.6	475.2	MT
Robin	38.9 c	47.6 de	86.0 c	100.3 d	36.7	35.6	682.5	629.7	MT
Njoro BW 2	36.7 c	40.0 g	80.3 cd	88.2 e	30.0	36.7	558.9	498.1	MT
Korongo	42.2 bc	45.6 def	91.5 bc	96.8 de	48.9	45.6	670.2	562.0	MT
Kenya Hawk	37.8 c	43.3 efg	79.4 cd	90.7 e	43.3	34.4	663.7	674.1	MT
Kenya Wren	46.7 b	45.6 def	98.6 b	94.6 de	32.2	38.9	465.3	535.6	MT
Kibis	37.8 c	42.2 fg	80.0 cd	89.2 e	36.7	47.8	496.2	442.7	MT
Chiriku	38.9 c	45.6 def	82.2 c	96.9 de	38.9	50.0	457	482.8	MT
957	42.0 bc	48.7 cd	90.7 bc	102.6 cd	44.4	52.2	650.3	600.3	S
4969	47.8 b	52.2 bc	101.0 b	110.7 bc	47.8	62.2	713.4	720.0	S
10155	53.3 a	56.7 a	113.5 a	120.1 ab	55.6	87.8	748.3	567.7	S
10213	42.2 bc	48.9 bcd	91.6 bc	103.1 cd	51.1	37.8	727.5	591.5	S
LSD (p ≤ 0.05)	5.6	4.4	11.0	8.6	15.1	16.4			
CV (%)	2.2	0.7	2.1	0.3	3.7	2.8			

Means followed by the same letter(s) within each column are not significantly different at $P \leq 0.05$; LSD: least significant difference; CV: coefficient of variation; MT - moderately tolerant, S - susceptible; AUDPC - area under disease progress curve.

Variety Kibis had low levels of DON but symptoms of FHB were more pronounced. Musyimi *et al.* (2012) reported Kibis as a susceptible variety. There was high infection of kernels of all the varieties as indicated by high re-isolation frequency of *F. graminearum* and high DON content. *Fusarium graminearum* causes greater visual symptoms and yield losses compared to other *Fusarium* spp. (Geddes *et al.*, 2008). Two types of resistance have been reported (Schroeder and Christensen, 1963); type I resistance to primary infection, usually measured by recording the number of infected spikelets 7 to 21 days after inoculation and type II resistance to disease spread, characterized by pathogen spread in infected spikes after point inoculation (Zhang *et al.*, 2011). Other types of resistance have been identified: type III, resistance to DON accumulation; type IV, resistance to kernel infection and type V, resistance to yield loss (Mesteharzy, 1995). *Fusarium* head blight is polygenic and the symptom expression is highly influenced by the environment (Sip *et al.*, 2008; Chrpova *et al.*, 2010). Resistance against FHB in small grain cereals is determined by several qualitative trait loci (Chrpova *et al.*, 2010).

Wheat lines and varieties evaluated in this study accumulated high levels of DON but the concentrations differed between the two sites and among the varieties and lines. The concentration in DON among the lines and varieties differed in the three sites. Varieties Kenya Wren, Kibis and Chiriku had the lowest levels of DON in the field while varieties Kibis, Chiriku and Njoro BW2 had the lowest levels in the greenhouse. Studies have shown that susceptible varieties accumulate more DON than resistant ones (Mesteharzy *et al.*, 2003; Cowger *et al.*, 2009; Wegulo, 2012). Variety Kwale showed moderate resistance to FHB and accumulated DON levels comparable to or higher than levels in susceptible varieties. This observation concurs with the findings by Hanarnandes-Noposa *et al.* (2010) who reported that a moderately resistant variety accumulated more toxins compared to susceptible ones. However,

a tolerant variety could be less suitable for DON production or may possess mechanisms that degrade DON (Muthomi, 2001; Mesteharzy *et al.*, 2003; Cowger *et al.*, 2009). The reason for high DON accumulation on a resistant variety is not known, however, Arseniuk *et al.* (1999) concluded that regulation of DON production in wheat may be independent of *Fusarium* head blight reaction. Varieties with high DON accumulation could have a dwarfing gene Rht-D1b (Abate *et al.*, 2008). Indeed, all varieties with high susceptibility to DON accumulation carry this gene (Chrpova *et al.*, 2013). Varieties with low levels of FHB have also been reported to accumulate high concentrations of mycotoxins (Mesterhazy, 1997). Therefore, not all lines or varieties with tolerance or resistance to FHB are necessarily resistant to mycotoxin accumulation.

Concentration of DON in samples from the greenhouse trial was lower compared to levels in samples from field trials. This variation could be attributed to the natural interplay between the pathogen and the environment thus providing a conducive atmosphere for disease expression and DON accumulation in the field. Environmental factors have been reported to affect DON levels during infection process (Merhej *et al.*, 2011; Wegulo, 2012). Humidity and high rainfall during and after anthesis result in increased DON production and more FHB symptoms (Landschoot *et al.*, 2012; Lindblad *et al.*, 2012). Favourable weather conditions are important during the vegetative wheat growth stage enhancing survival of the primary inocula present in the soil and plant debris (Landschoot *et al.*, 2012).

Late maturity of variety Kwale may have contributed to the high DON accumulation in the grain. Wegulo *et al.* (2011) working with a moderately resistant variety Harry reported higher *Fusarium* damaged kernels and DON accumulation. Deoxynivalenol is a potent inhibitor of protein biosynthesis that affects digestive system and function of major organs in humans and animals (Afsah-Hejri *et al.*, 2013). When taken with food or feed, it results in nausea, vomiting, and diarrhoea (Harnandes-Noposa, 2010).

Farm animals fed with DON contaminated feed are characterized by reduced weight and feed refusal. Because of the health implications of DON, tolerance limits have been set at 0.001 ng/g, 0.01 ng/g, and 0.005 ng/g in finished products, grain and grain by products, respectively. Contamination of grains with DON is highest when infection occurs at mid anthesis (Lacey *et al.*, 1999).

Severity and AUDPC of FHB were positively correlated to re-isolation of *F. graminearum* indicating that any of the parameters can be used to assess varietal resistance as reported by Muthomi (2001). The relationship between visual symptoms of FHB and DON content is highly variable ranging from none to a very strong positive relationship (Paul *et al.*, 2005). The difference in relationships may be due to differences among wheat varieties, weather conditions, pathogen population and disease management practices (Harnandes-Nopsa, 2010). Therefore, FHB symptoms cannot be used to predict the level of accumulated DON in the resulting grain (Bai *et al.*, 2001). In the current study, level of deoxynivalenol was correlated to FHB incidence, severity and AUDPC in both field trials. Liu *et al.* (2013) working on molecular characterization of soft red winter wheat found flowering time and heading positively correlated with DON levels indicating that genotypes with early heading and flowering times escaped the optimal favorable infection conditions that resulted in lower DON levels.

The association between FHB intensity and DON in small grain cereals has been extensively studied (Wegulo *et al.*, 2012). Understanding this relationship can be used to develop predictive models which can be used to manage FHB. It has also been shown that correlations between FHB intensity can be affected by wheat type and study location (Paul *et al.*, 2005). Significant correlation between visual estimate of the disease severity and DON concentration was also reported by Usele *et al.* (2013). High and positive correlation between disease severity in the field and the concentration of DON in grain has been reported by several researchers (Zhu *et al.*, 1999; Buerstmayr *et al.*, 2004; Usele *et al.*, 2013).

This allows a breeder to choose lines with lower FHB severity and at the same time with low mycotoxin accumulation in grain. Varieties Kwale, Njoro BW2, Kibis, and Kenya Hawk were found to be moderately tolerant to FHB and are therefore recommended for farmers in Kenya. This study demonstrated that disease and tolerance to toxin accumulation are useful criteria in breeding and should be combined to achieve optimum performance.

Acknowledgement

This project was funded by the Regional Universities Forum for Capacity Building in Agriculture (RUFORUM), Grant No. RU/2012/GRG-69.

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