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Reaction of Some Kenyan Wheat Cultivars to Head Blight after Inoculation with *Fusarium graminearum*

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Abstract: Eight cultivars of wheat that are commonly grown in Nakuru and Nyandarua districts of Kenya were tested for their susceptibility to *Fusarium* head blight (scab) under green house conditions. The cultivars were inoculated with mixed inoculum derived from three pathogenic isolates of *Fusarium graminearum* that had previously been isolated from wheat. Head blight severity was assessed using a 1-9 scale based on proportion of spikelets bleached and the area under disease progress curve was derived from the disease severity data. At harvest, kernel weight reduction as compared to the untreated controls was determined. All the 8 wheat cultivars were found to be susceptible but they differed in the level of susceptibility. Disease severity among the cultivars varied from 5-59% while the area under disease progress curve varied from 93-994. Cultivars 'mbuni' and 'chiriku' were the most susceptible, with a grain weight reduction of up to 74%. The results indicate that most of the varieties grown in Nakuru and Nyandarua districts are susceptible to *Fusarium* head blight. The study indicated that Njoro Bw1 and Njoro Bw2 are the most promising. The resistance trait in these varieties could be useful in *Fusarium* head blight management.

Key words: *Fusarium*, head blight, wheat, susceptibility

INTRODUCTION

Fusarium Head Blight (FHB) or (scab) of wheat is a common disease of wheat, barley and oats and can result in a reduction in grain quality and yield (Parry *et al*., 1995). The significance of the disease in wheat production is attributed to both yield reduction and mycotoxin contamination of the grain harvested from the infected ears. The disease is caused by different *Fusarium* species, including *F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. poae*, *F. crookwellense*, *F. sporotrichioides* and *Macrodochium nivale* (Parry *et al*., 1995). *Fusarium graminearum* and *F. culmorum* are the most virulent, causing severe blighting of wheat ears (Manka *et al*., 1985; Stack and McMullen, 1985). Several outbreaks of severe on wheat, mainly associated with *F. graminearum*, have been experienced since 1990 in different parts of the world. Economic losses in USA were estimated at over $2.5 billion during 1992-1998 (Windels, 2000) and $2.7 billion in 1998-2000 (Nganje *et al*., 2004). *Fusarium graminearum* also infects maize causing pink ear rot (Sutton, 1982). The fungi can infect all the vegetative and reproductive parts of cereal crops and survives in crop residues (Jones, 2000).

Fusarium species that infect cereals inhabit soils and are capable of surviving saprophytically on crop debris (Jones, 2000; Parry *et al*., 1995) and ploughing to burry crop debris removes the source of inoculum from the soil surface which could be available for dispersal to ears. Pathogen population in wheat field soils fluctuate throughout the season, increasing during dry conditions that favour pathogen activity on stem bases (Goswami and Kistler, 2004; Bateman and Murry, 2001; Bateman *et al*., 1998; Vigier *et al*., 1997). Conidia are dispersed either by wind or rain splash to wheat heads (Fernado *et al*., 1997; Jenkinson and Parry, 1994). There is no evidence that seedborne inoculum contributes to *Fusarium* head blight (Bateman, 2005) but the pathogen may be dispersed by seed (Mishraa *et al*., 2002). The conidia infect ears mainly during anthesis (Bai and Shanner, 1996). The success of infection depends on weather conditions such as temperature (Brennan *et al*., 2005; Cowger, 2005; Stein *et al*., 2005; De Wolf, 2003; Mentewab *et al*., 2000), humidity (Cowger, 2005; Nita *et al*., 2005), cultivar resistance (Cowger, 2005; Nita *et al*., 2005; Llorens *et al*., 2004) and nitrogen fertilization (Doohan *et al*., 2003). However, it is the availability of moisture that is the overriding factor (Lacey *et al*., 1999).

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Management options include cultural practices, use of fungicides, breeding for resistance and biological control. Cultural management options include crop rotation, appropriate use of fertilizers, irrigation, weed control, proper land preparation and timely harvesting. Minimum tillage and continuous cereal cropping increases the incidence and severity of *Fusarium* head blight (Pereyra and Dill-Macky, 2004). It is critical to avoid planting wheat adjacent to fields with large amounts of small grain or maize residues remaining on the soil surface. No till planting of wheat into maize residues substantially increases *Fusarium* head blight infection. Rotation with a legume crop between maize and small grain crops provides time for the residues to break down and the pathogen population to decline (Champeil *et al*., 2004). Control of *Fusarium* head blight using fungicides has been reported to give inconsistent results due to the complexity of the causal organism, influence of nitrogen fertilization, timing of fungicide application and masking control of one *Fusarium* species by the subsequent growth of another species (Heier *et al*., 2005; Ramirez *et al*., 2004; Magan *et al*., 2002; McMullen *et al*., 1997; Parry *et al*., 1995). Application of some fungicides has been shown to stimulate deoxynivalenol and nivalenol production particularly at sub-optimal fungal growth conditions and low fungicide dosage (Ramirez *et al*., 2004; Magan *et al*., 2002; Jennings *et al*., 2000; D'Mello *et al*., 1999). In addition, food safety concerns limit the chemical management options due to fungicide residues in grain and wheat products (Magan and Olsen, 2004; Jones, 2000).

Most wheat cultivars available are susceptible to *Fusarium* head blight, none are immune and only a few are moderately resistant (Muthomi *et al*., 2002a; Parry *et al*., 1995). However, the use of resistant cultivars still remains the most effective, environmentally friendly, economical and safe strategy of containing the disease and associated mycotoxin contamination. Significant levels of partial resistance that limit yield loss and mycotoxin accumulation have been reported (Pereyra and Dill-Macky, 2004; Wisniewska and Kowalczk, 2005; Miedaner, 1997). Sources of resistance have been found in China, South America and Czech Republic (Lin *et al*., 2006; Mesterhazy *et al*., 1999; Mesterhazy, 1995). Wheat varieties exhibit non-specific resistance to head blight due to the large number of *Fusarium* species involved and lack of genes for complete resistance.

In this study, 8 wheat varieties commonly grown in Nyandarua and Nakuru Districts of Kenya were challenged with *Fusarium graminearum* under green house conditions with the objective of determining their susceptibility to *Fusarium* head blight based on symptom expression and yield reduction.

MATERIALS AND METHODS

Survey and plant cultivation: A total of 90 farms were surveyed in Nakuru and Nyandarua districts of Kenya for wheat cultivars grown during the 2004 growing season. The farmers were randomly selected to cover 50 farms in Nakuru and 40 farms in Nyandarua district. For the greenhouse inoculation experiment, certified seeds of 8 wheat varieties (Kwale, Mbuni, Njoro Bw1, Njoro Bw2, Heroe, Chiriku, Chozi and Yombi) were acquired from Kenya Agricultural Research Institute (KARI)-Njoro. Each cultivar was planted in pots (25 seeds pot^{-1}) in forest soil-farm yard manure medium (2:1 v/v). To ensure that flowering dates for all the varieties were synchronized, the late maturing varieties were planted earlier than the early maturing varieties. The plants were maintained outside the green house until flowering (GS61, Zadoks *et al*., 1974) to simulate field conditions. The plants were fertilized after germination at GS10 (NPK 20:20:0), at tillering GS22 (NPK 20:20:0) and at booting GS41 (urea 46% N) at the rate of 5 g pot^{-1}. Leaf rust (*Puccinia recondite*) was controlled by spraying with triadimefon (Bayleton®) at tillering (GS 22) at the rate of 1 g L^{-1} while leaf chewing insects and aphids were controlled with dimethoate (Danadin® 40 EC) at the rate of 1 mL^{-1}. The experiment was repeated over two greenhouse cropping cycles.

Inoculum production and inoculation: *Fusarium graminearum* was isolated from infected wheat seeds obtained from farmers fields during the 2004 cropping season. The seeds were surface sterilized for 3 min in 5% sodium hypochlorite containing four drops of 20 for 3 min then rinsed three times with distilled water. The kernels were plated on low strength Potato Dextrose Agar (PDA) media amended with mineral salts (Muthomi, 2001). The *Fusarium* isolates were identified to species level based on synoptic keys by Nelson *et al*. (1983). Pathogenicity of the different isolates was tested by inculation onto ears of a susceptible wheat variety (Mbuni) according to Muthomi *et al*. (2002b). Three most pathogenic isolates of *Fusarium graminearum* were selected for the study. Each isolate was cultured separately on potato dextrose agar and inoculum was multiplied in mung bean liquor medium (Bai and Shaner, 1996). Spore suspension for each isolate was prepared by filtering the aqueous culture through

two layers of cheesecloth and spore concentration adjusted to 5×10^5 spores mL^{-1}. The spore suspensions of the 3 isolates were then mixed to make a composite inoculum and a few drops of 20 added to ensure uniform spore dispersion. Inoculation was done at 50% flowering (GS65) by spraying the ears with the spore suspension using a hand sprayer. Re-inoculation was done after 4 days. Control pots were sprayed with distilled water. The inoculated ears were immediately covered with polythene bags for 48 h to ensure high humidity for infection. Four pots were inoculated per cultivar and the experiment arranged in a complete randomized block design. After inoculation, the plants were allowed to mature inside the greenhouse (22±5°C) to ensure uniform environmental conditions and to avoid damage from birds.

Head blight assessment and effect on grain yield: Head blight severity was assessed visually as the proportion of bleached spikelets based on a 1-9 scale (Miedaner and Perkowski, 1996), where 1= no symptoms, 2 = <5%, 3 = 5-15%, 4 = 16-25%, 5 = 26- 45%, 6 = 46-65%, 7 = 66-85%, 8 = 86-95% and 9 = 96-100% of bleached spikelets. Ten average sized ears per pot were tagged and assessed for disease every 5th day starting with onset of symptoms until yellow ripening (GS87). The mean disease severity value for each replicate pot was calculated by averaging the disease severity values for the different assessment times. The Area Under Disease Progress Curve (AUDPC) was calculated as follows (Shaner and Finney, 1977):

$$AUDPC = \sum [(Y_{i+1} + Y_i)/2] [t_i+1 - t_i],$$

Where, Y_i is the disease severity at the ith observation, n is the total number of observations, t_i is the time (days) at the ith observation. At maturity, the tagged ears were harvested separately for the different cultivars, dried and threshed by hand for grain weight determination. Seed grains of each cultivar were weighed separately (ten ears per pot). Data was subjected to analysis of variance (ANOVA) using Genstat® for Windows, 6th edition. Where treatment effect was significant, pair-wise treatment mean differences were determined by Tukey Least Significant Difference (LSD) test at 95% confidence limit.

RESULTS

The most widely grown wheat cultivars in the two districts surveyed during the 2004-growing season were Kwale, Mbuni, Chiriku, Nyangumi and Pasa (Table 1). The 8 cultivars challenged with *Fusarium* head blight by inoculation with *F. graminearum* significantly differed ($p\leq0.05$) in both disease severity and area under disease progress curve (Table 2). The mean disease severity ranged from 9.0% for the least susceptible (Njoro Bw2) to 51.6% for the most susceptible cultivar (Mbuni). The area under disease progress curve varied from 140.5 to 956 for the least and most susceptible cultivars, respectively. Disease severity was highly significantly ($p\leq0.05$) correlated to the area under the disease progress curve ($r = 0.85$ to 0.99). The cultivars also significantly differed ($p\leq0.05$) in grain weight. The most susceptible cultivars were Mbuni, Chiriku, Yombi, Kwale and Chozi while the least susceptible were Njoro Bw2, Heroe and Njoro Bw1 (Fig. 1). Based on head blight severity and the area under disease progress curve, the 8 wheat cultivars could be grouped into three susceptibility groups (Fig. 1): susceptible (Mbuni and Chiriku), moderately tolerant (Yombi, Kwale and Chozi) and tolerant (Njoro Bw1, Heroe and Njoro Bw2). In the most susceptible cultivars, symptoms appeared as early as 5 days after inoculation and disease development was faster compared to 10 days incubation period for the least susceptible cultivars (Table 3). The most widely grown cultivars in Nakuru and Nyandarua districts, Kenya, were among the highly susceptible and moderately susceptible.

Table 1: Percentage of farmers growing different wheat cultivars in Nakuru and Nyandarua districts of Kenya during the 2004 cropping season

Cultivars	Nakuru	Nyandarua	Overall mean
Kwale	23.0	25.5	23.3
Mbuni	19.7	14.9	17.3
Chiriku	9.8	15.0	12.4
Nyangumi	11.5	4.3	7.9
Pasa	0.0	12.8	6.4
Njoro Bw2	8.2	0.0	4.1
Heroe	8.2	0.0	4.1
Kongoni	0.0	8.5	4.3
Nduma	3.3	4.3	3.8
NjoroBw1	4.9	0.0	2.5
Mbega	4.9	0.0	2.5
Tembo	0.0	6.4	3.2
Ngamia	0.0	4.3	2.2
Fahari	1.6	2.1	1.9
Others*	4.9	2.1	3.5

* Mwamba, Chozi, Popo, Royal

Table 2: Mean disease severity, 10 ear seed weight (g), 100 seed weight (g) and kernel weight reduction for the 8 wheat cultivars inoculated with *Fusarium graminearum*

Cultivars	% spikelets bleached	AUDPC	10 ear weight	% kernel weight reduction
Mbuni	51.6	956.0	4.0	26.0
Chiriku	48.6	908.0	3.2	50.5
Yombi	39.1	685.5	5.0	17.0
Kwale	33.3	567.0	2.6	41.0
Chozi	27.0	451.0	4.1	30.5
Njoro Bw1	23.1	385.5	4.2	40.5
Njoro Bw2	9.0	140.5	3.6	53.5
LSD ($p\leq0.05$)	8.7	174.7	1.5	8.7

AUDPC = Area Under Disease Progress Curve

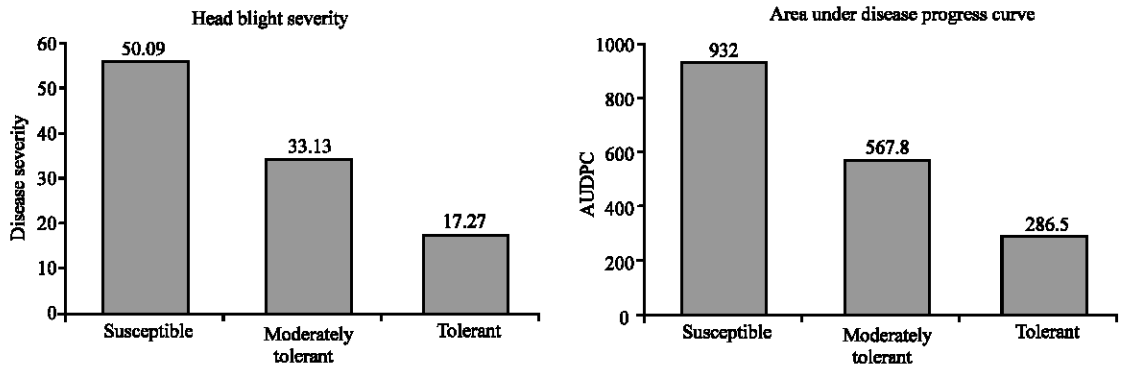

Fig. 1: Susceptibility groups of wheat cultivars based on the mean head blight severity and the area under disease progress curve (Susceptible-Mbuni and Chiriku; Moderately tolerant-Yombi, Kwale and Chozi; Tolearant- Njoro Bw1, Heroe and Njoro Bw2)

Table 3: Disease progress on 8 different wheat cultivars inoculated with *Fusarium graminearum*

	Days after inoculation						
Cultivars	0	10	15	20	25	30	Mean
Mbuni	0.0a	6.3a	30.5a	49.3a	60.5a	72.5a	36.5
Chiriku	0.0a	7.2a	26.7ab	50.0a	56.0a	69.9a	35.0
Yombi	0.0a	0.6a	17.0bc	30.1b	44.4b	62.3ab	25.7
Kwale	0.0a	0.8a	6.0d	14.2c	37.0bc	64.8a	20.4
Chozi	0.0a	1.2a	2.5d	10.6cd	34.8bcd	63.0ab	18.7
Heroe	0.0a	1.3a	4.7d	13.1cd	31.0cd	52.3bc	17.1
Njoro Bw 1	0.0a	0.7a	7.4cd	15.2c	24.3d	50.3c	16.3
Njoro Bw 2	0.0a	0.0a	0.5d	3.3d	8.0e	13.8d	4.3

Means followed by same letter(s) within columns are not significantly different (p = 0.05)

DISCUSSION

The results indicated that the widely grown wheat cultivars in Nakuru and Nyandarua districts of Kenya were susceptible to *Fusarium* head blight. This suggests that head blight could pose a major threat to wheat production in the two districts in case an epidemic broke out under favourable weather conditions. All the 8 wheat cultivars that were inoculated with *Fusarium graminearum* were found to be susceptible to head blight. However, the cultivars differed in susceptibility as indicated by the incubation period, disease severity and kernel weight reduction. The results were consistent with findings by Muthomi *et al.* (2002a) who tested 15 different Kenyan wheat cultivars and found that they were all susceptible to *Fusarium* head blight. The cultivars could be divided into three susceptibility groups: highly susceptible (Mbuni and Chiriku), moderately susceptible (Yombi, Heroe, Chozi, Kwale and Njoro Bw1) and moderately resistant (Njoro Bw2). The differences in susceptibility among the cultivars could probably be due to inherent genetic resistance factors.

Two types of resistance to FHB of wheat have been reported; resistance to primary infection and resistance to spread of the disease within a spike (Lin *et al.*, 2006; Ma *et al.*, 2006; Wang and miller, 1988; Schroeder and Christensen, 1963). A third mechanism, resistance to deoxynivalenol accumulation has been proposed (Ma *et al.*, 2006; Miller *et al.*, 1985) and deoxynivalenol may play a role in *Fusarium* head blight pathogenesis (Snijders and Krechting, 1992). *Fusarium* head blight resistance in wheat has been reported to be a quantitative trait that is controlled by a polygenic system. Effects of dominance of genes probably influence FHB resistance, but additive effects appear to be important and resistance genes can be accumulated (Shen and Ohm, 2006; Bai *et al.*, 1999). Chromosomal location of FHB resistance genes/loci in the wheat genome has been mapped using RFLP and AFLP methods and recombinant inbred lines. Quantitative trait loci of resistance to FHB have been preliminarily mapped on the following chromosomes: 1B, 2A; 2AL, 2B, 3 BS, 3A, 3B, 3D, 4A, 4B, 5A, 5B, 5D, 6A, 6B and 6D (Lin *et al.*, 2006; Ma *et al.*, 2006; Shen and Ohm, 2006; Mardi *et al.*, 2005; Shen *et al.*, 2003). Growing of wheat cultivars resistant to *Fusarium* sp. is the most economic, environment-friendly and effective method of disease control. Sources of resistance have been found in China, South America and Czech Republic (Lin *et al.*, 2006; Mersterhazy *et al.*, 1999; Mersterhazy, 1995). Currently, there are no wheat varieties with high level of resistance to FHB although some varieties have useable levels of partial resistance that limit yield loss and mycotoxins accumulation (Shen and Ohml, 2006; Pereyra and Dill-Macky, 2004). Cultivar Sumai 3 from China is reported to be the best source of resistance (Kolb *et al.*, 2001) and its quantitative trait loci located on the distal end of the short arm of chromosome 3B (Liu *et al.*, 2005) is stably expressed in different environments and in different genetic backgrounds (Shen *et al.*, 2003; Zhou *et al.*, 2002).

It combines good resistance to both type I and type II infection and gives one of the lowest levels of DON in the kernels following infection. Good resistance to FHB can also be found in wild relatives of wheat and related triticale species. A recent study by Shen and Ohm (2006) reported that FHB resistance on chromosome 7E of wheatgrass *Lonhopyrum elongatum* confers a large effect and has an additive effect with chromosome 3B of chinese wheat cultivar Sumai 3. Such incorporation of FHB resistance from related species is critical to improving the level of resistance, enhancing genetic diversity and providing continuing resistance.

This study could be useful in providing information to wheat producers in wheat growing areas of Kenya on selection of cultivar during planting. This is critical considering that most of the cultivars grown in Nakuru and Nyandarua districts (as well as other wheat producing regions in Kenya) are susceptible to FHB. Therefore the selected cultivar will among other factors determine disease infection levels and possible mycotoxin contamination besides the economic implications of FHB management or resultant yield loss. The study also highlights the need for continued breeding for resistance against FHB causing fusaria. Further studies are needed to determine the type of resistance in local wheat cultivars and to determine whether the genes involved could be useful in development of new cultivars.

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