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Evaluation of Response of Wheat (Triticum spp) Genotypes against Wheat Fusarium Head Blight in Ethiopia

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Abstract

Fusarium head blight, or head scab is a destructive disease of wheat, maize and other small grain cereals. It is predominantly incited by *Fusarium graminearum* worldwide. In Ethiopia, the disease becoming an important biotic constraint for wheat production and this study was aimed to determine the reaction of some released Ethiopian wheat varieties to the disease. A total of 38 Ethiopian wheat varieties (30 bread and 8 durum types) were screened for the disease resistance at Ambo Agricultural Research Center during the 2019/2020 season. The varieties were artificially sprayed twice (at 50 and 100% flowering stage) using hand sprayer with *F. graminearum* inoculum isolated from infected grains of wheat. The result showed that 31(81.6%) tested varieties exhibited susceptible reaction, while only 7 (18.4%) varieties, namely Enkoy, Huluka, Galema, Hogana, K6295-4A, Dashen and Derselegne showed moderately tolerant reaction to the disease. The disease is known to cause yield losses as high as 58.20% on susceptible wheat variety (Ogolchu) assessed in this study. Hence, the study concludes that the disease pose threat to wheat production and most of wheat varieties currently under production in the country were among the susceptible varieties. Therefore, breeders should focus on the improvement of the crop through developing multiple and race non-specific resistance varieties to the disease. Massive screening of introduced and locally available wheat genotypes including those evaluated in this study over years and locations is also needed. The identified moderately tolerant varieties by this study should be used with proper agronomic practices and other possible disease management options.

Keywords: Fusarium head blight; Fusarium graminearum; Wheat; Resistance

Introduction

Fusarium Head Blight (FHB), or head scab, is a destructive disease of maize, wheat and other small grain cereals in wet and humid areas worldwide. Even though it is incited by complex *Fusarium* species, the disease is predominantly caused by *Fusarium graminearum* in many countries. The disease is sporadic in nature and, the distribution and intensity of these pathogens highly depends on environmental factors. Severe epidemics of the disease are occurred when virulent strains of these pathogens are coincided with favourable environmental conditions and susceptible host with vulnerable crop growth stage. The disease mostly affects wheat heads and it is known by causing both quantity and quality losses.

In Ethiopia, it is becoming an important biotic factor that limits wheat production and productivity in wheat growing areas. Recently, the survey results conducted in the country revealed that the disease problem has been increased unusually due to weather condition fluctuation, improper agronomic practices and other factors increasing the disease epidemics in the area. Additionally, various reports in the country indicated as the disease epidemics are also highly increased on wheat production during offseason.

The management options so far recommended for the control disease include cultural practices, cultivar resistance, application of fungicides and integrated management. The use of resistant/tolerant varieties against complex *Fusarium* species is still remains the most effective, durable, environmentally safe and economically feasible strategy for managing the disease and associated mycotoxin contamination. However, the reaction of released Ethiopian wheat varieties to the disease and searching for new resistance sources are not well studied. Therefore, this study was undertaken to determine the reaction of some released Ethiopian wheat varieties to the disease in Western Shewa Zone, Oromia Regional state of Ethiopia [1].

Materials and Methods

Description of the Study Areas and Materials

The study was conducted during 2019 cropping season at Ambo Agricultural Research Center (AmARC) pilot site, which is located at 8° 57' 58" N latitude and 37° 51' 33" E longitudes and at an altitude of 2175 m.a.s.l. The mean annual rainfall, minimum and maximum temperature of the study area during the experimental period were, respectively, 1265.7mm, 11.7°C and 25.6°C. The soil type of AmARC is vertisol with a pH value of 6.8. Thirty-eight released Ethiopian wheat varieties (30 bread and 8 durum wheat types) obtained from Kulumsa and Debrezeit Agricultural Research Centers of Ethiopian Institute of Agricultural Research (EIAR) were screened for Type I and Type II FHB resistance following spray inoculation under field condition. Each variety was sown in two (inoculated and uninoculated) plots of 1m x 1m = 1m^2, with each plot having five rows and 1m between plots. Flowering dates of 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. Inorganic fertilizer (NPS & Urea) and all agronomic practices were applied according to the recommendations [2].

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Inoculum Preparation and Plant Inoculation

F. graminearum inoculum was isolated from infected wheat grains collected from AmARC research fields during 2018 cropping season. The kernel samples were surface sterilized in 5% sodium hypochlorite for 3 minutes and then rinsed thrice with sterilized distilled water. The sterilized kernels were allowed to air dry under hood and then plated on PDA medium that amended with Chloramphenicol antibiotics. The cultured samples were incubated at 27°C temperature for five days and purified by sub culturing the grown fungal pathogens on fresh PDA media with the help of a sterile loop to obtain pure isolates of the pathogens for identification. The Fusarium isolates were identified to species level based on synoptic keys (1983). The identified *F. graminearum* was used for pathogenicity test to get virulent pathogen by inoculating isolates on early mature and susceptible variety (Kakaba variety). This variety was highly devastated by the disease during 2018 season at AmARC field station. Two most pathogenic isolates of *F.graminearum* were selected based on pathogenicity test result under greenhouse condition. The selected pathogens were re-isolated and purified separately on PDA medium [3].

The seven days old cultures were then transferred to Mung Bean Broth Media (MBBM) for five days to produce enough macroconidia for inoculation. The mung bean medium was prepared by boiling 40g of mung bean in 1000ml of water for 15 minutes. The extract was filtered through cheese cloth and a 100ml portion of the extract was autoclaved at 121°C for 20 minutes at 15psi. The mung bean extract was cooled in an Erlenmeyer flask and inoculated with purified seven-day old *F. graminearum* isolate grown on PDA. Then it was 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.

The suspensions of the two selected virulent isolates were filtered by using cheese cloth and they mixed-up to obtain the isolate pool used for artificial inoculation. Then the conidial suspension was adjusted to 1×10^5 conidia ml^{-1}. The Tween 20 was added to the suspensions at 0.05% (v/v) concentration to ensure uniform dispersion was used for artificial inoculation. The spore suspension was sprayed twice at 50% and 100% anthesis wheat growth stage (GS 64/65), the stage at which wheat is most susceptible to the disease. Control plots were sprayed with sterile distilled water containing 0.05% (v/v) Tween 20. The inoculated spikes were covered with a polythene bag for 48 hours to ensure high relative humidity for optimal infection to take place [4].

Disease assessment

Disease Incidence (%): Determined as proportion of infected plants showing blighted symptoms in each plot.

$$DI(\%) = \frac{\text{Number of Diseased plants}}{\text{Total number of plants observed}} * 100$$

Disease severity (%) : Was estimated based on the percentage of the blighted spikes of the randomly tagged ten plants from the middle three rows of each plot. It was collected five times (7, 12, 17, 22 and 27 days after inoculation). Ten average sized spikes from each plot were tagged and assessed five times every 5 days interval (at 7, 12, 17, 22 and 27 days) after inoculation for disease severity until yellow ripening (GS87). The disease severity was rated based on a 1-9 scale, where: 1 = no symptoms, 2 = <5%, 3 = 5-15%, 4 = 16-25%, 5 = 26-45%, 6 = 46-65%, 7 = 66-85%, 8 = 86-95%, 9 = 96-100% of spikelets bleached. Severity scores were converted to percent of disease severity index, (PSI) using the formula suggested by Wheeler (1969) and Kumar *et al.* (2011) as described below.

$$PSI(\%) = \frac{\text{Sum of numerical rating}}{\text{Total number of plant observed x maximum rating}} * 100$$

Area Under Disease Progress Curve (AUDPC) was calculated by using severity scale which were collected from each plot every time intervals using the formula developed by Shanner and Finney (1977).

$$AUDPC = \sum_{i-1}^{n-1} 0.5[(x_{i+1} + x_i)(t_{i+1} - t_i)]$$

Where, X_i is the cumulative disease severity expressed as a proportion at the i^{th} observation; t_i is the time (days after inoculation) at the i^{th} observation and n is total number of observations.

Agronomic data such as plant height, spike length, number of kernels per spike, plant biomass, Fusarium Damaged Kernel (FDK), thousand seed weights and grain yield per plot and per hectare were recorded from the middle three rows of each experimental unit.

Data analysis

The collected experiment data analysis was done to determine the significance of the effect of the two treatments (infected and uninfected) on yield and yield components using t-test. Pearson correlation analysis were calculated using the PROC CORR function SAS program version 9.4 to correlate disease intensity with yield and yield components [5].

Result and Discussion

Disease intensity, Incubation period and Response of varieties to FHB

The evaluated both bread and durum wheat types for their reaction to FHB under field condition by using spray inoculation with virulent *F. graminearum* inoculum exhibited different reaction to the disease. The premature bleaching/blighting of head symptoms was developed on all inoculated wheat varieties at different time (incubation period) and disease extent (Figure 1). However, uninoculated plots remains healthy without developing the disease symptom. The disease symptom was developed as early as 5 days post inoculation (PI) on most susceptible varieties and after 7-12 days PI on susceptible and relatively moderate susceptible varieties. The symptom was appeared early on Kakaba, Lemu, Danda'a, Millennium, Tesfaye, Utuba, Honkolo, Ogolcho, Ude, Hidase and Mukiye varieties and later on Enkoy, K6295-4A, Don Mathew and Digelu varieties.

The present results suggest that the shorter incubation period of the pathogen for symptom development showed the more susceptibility

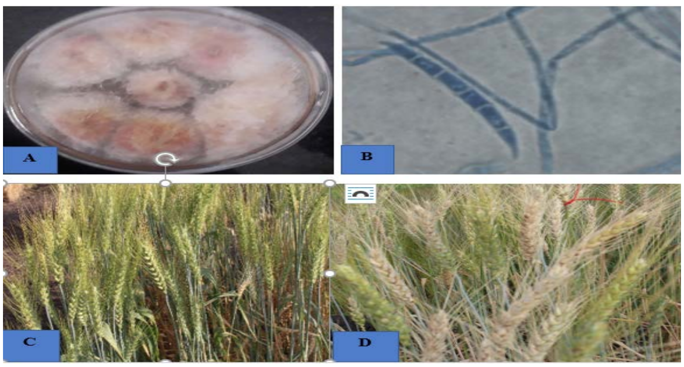

Figure 1: The seven day mycellium **(a)** of F.gramenearum mycelium grown on PDA medium and its conidia **(b)** detected under compound microscope(b),uninoculated plot **(c)** and FHB symptom developed on inoculated plots**(d).**

of the variety to the disease and vice versa. Since the susceptible varieties unable to hinder the growth of pathogen, the pathogen grows fastly in/on them within short period of time. The delayed symptom development in heads of some wheat varieties indicated as the variety restricted the development of pathogen in some extent and showed their tolerant level to the disease. These findings are consistent with Browne and Cooke (2005) who reported that the incubation period is delayed and latent periods are longer in in resistance varieties or crops than in susceptible once [6].

Even though the disease symptom was appeared in all inoculated varieties, the disease incidence, severity and AUDPC was varied from variety to variety. The mean disease incidence was ranged from 10% for least susceptible (Enkoy, Galema, and Dereselegne) varieties to 75% for most susceptible (Danda'a) variety. However, the percent of disease severity index was ranged from 21% for the least susceptible (Galema) variety to 85% for the most susceptible (Kakaba) variety. More than 41.0% of the evaluated cultivars were showed more than 50% disease incidence. This indicated as majority of the evaluated varieties were failed to initial infection resistance which is used to determine type I resistance. Miedaner showed that the type I FHB is the resistance to the initial infection and evaluated based on FHB disease incidence following inoculation. However, the type II resistance is resistance to spread of pathogen with in spikes and evaluated based on disease severity [7].

The area under progress curve calculated from disease severity collected five times (at 7, 12, 17, 22 and 27 days) after inoculation was also varied from 217.5 to 1182 for the least susceptible (Galema) and most susceptible (Kakaba) varieties. The disease spread with in spikes of variety was varied according to variety and developed different disease progress curve as presented in (Figure 2). The highest AUDPC, indicated the dominance the *disease* in/on the infected parts with time and it showed inability of cultivar to inhibit the disease spread in the tissue. Grausgruber also described as the greater the AUDPC, is the more susceptible the variety or line to the disease.

Response of Wheat Varieties for their Reaction to FHB

Based on collected data, the evaluated varieties could be categorized in to three resistant levels as highly susceptible, susceptible and moderately tolerant. Of the screened wheat varieties, 7(18.4%) varieties namely: Enkoy, Huluka, Galema, Hogana, K6295-4A, Dashen and Dereselign) were found moderately resistant. However, 25(65.8%) varieties and 6(15.8%) varieties were exhibited susceptible and highly susceptible, respectively. None of the tested variety showed completely resistant to the disease. Since all varieties were tested under the same environmental condition and inoculum dose, differential response to FHB possibly reflect the presence of variation in the genetic makeup of varieties. Previously, Bekele (1990) reported Enkoy variety as moderately tolerant and Dashen variety as susceptible to the disease. In this study variety Dashen exhibited moderate tolerant reaction to the disease. This could be due to genotype-environmental interaction or disease escape (Harnandes-Nopsa, 2010). The result indicated the most currently cultivated wheat varieties by farmers in the study area are among the susceptible and highly susceptible varieties identified in this study. This suggests that as FHB could pose threat to wheat production in these areas due to favourable environmental conditions for the disease epidemics and mono cropping system practiced by farmers in the area.

The seven varieties identified by this study as moderately tolerant reaction to FHB were bread wheat types and all the durum wheat types included in this study exhibited susceptible and highly susceptible reaction to the disease (Figure 3). This might be due to tetraploid nature and limited number of durum wheat varieties and lack resistance materials found in this wheat types when compared to hexaploid wheat types. Many scholars similarly reported as durum wheat types are more susceptible than bread wheat types due to limited sources of FHB resistance gene (s) available in this wheat types. Likewise, Haile also stated durum wheat types are highly susceptible to FHB than bread wheat types and breeding this wheat type for FHB resistance is difficult due to their lack of resistance sources [8].

Effect of FHB on Yield and Yield Components of Wheat

The analyzed yield and yield related data collected from randomly selected 50 plants from each plot indicated there were significant difference ($P \leq 0.05$) between yields, plant biomass, kernel number per spike, and thousand kernel weight of healthy and diseased plants as presented. The highest yield loss (58.2%) due to the disease on

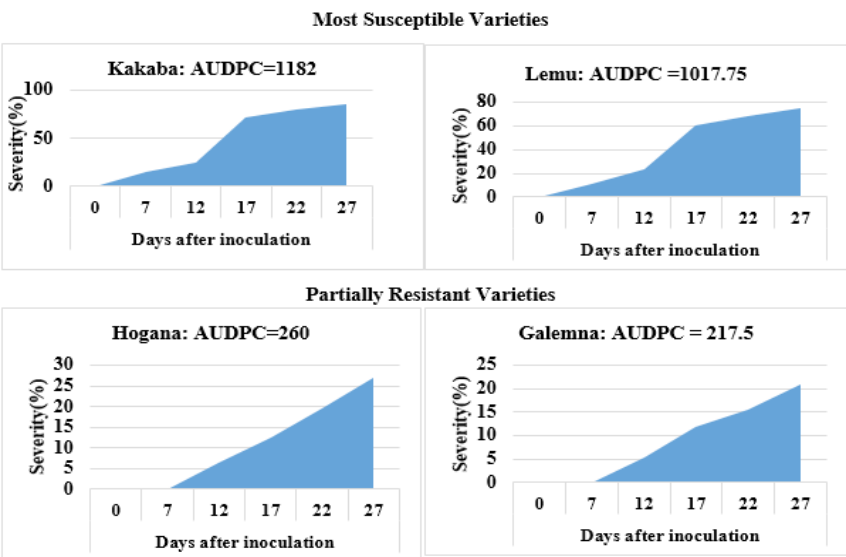

Figure 2: Differences in the shape of disease progress curves of mostly susceptible and partially resistant evaluated Ethiopian wheat varieties after 7,12,17,22 and 27 days of inoculation with *F. graminearum*

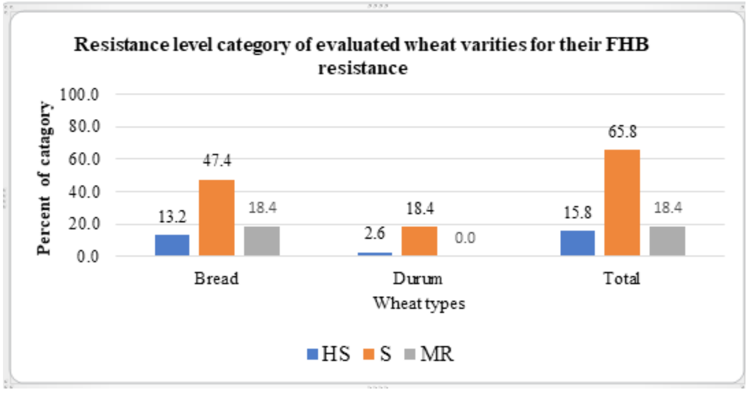

Figure 3: Proportion of the evaluated wheat varieties in different susceptibility level to FHB.

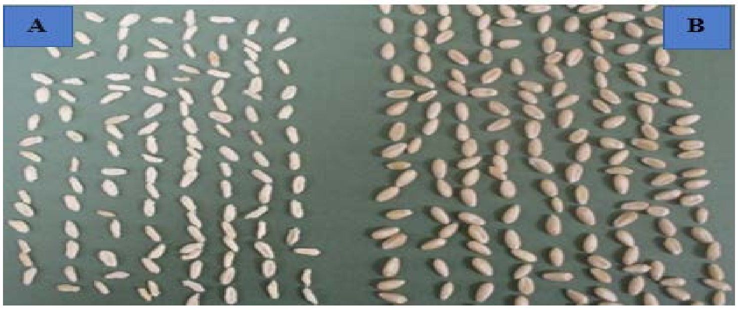

Figure 4: The white shriveled kernels harvested from infected plots(A) and healthy seeds that harvested from control plots (B).

susceptible variety Ogolchu was noted, and it is followed by Mangudo (56.3%), Kakaba (54.8%) and Lemu (52.5%) varieties, whereas the lowest yield reduction was recorded on variety Enkoy (18.1%). This variety was among varieties that had minimum disease severity which ranked as moderately tolerant to FHB in this study. The small, thin/shrunken, soft-whitened grains, or Fusarium damaged kernel (FDK) which were unfit for market as well as for consumption were harvested from highly infected plots by the disease as indicated. However, some spikes were left to bear grains due to the pollination abortion and spikelet sterility might be occurred by the disease and this condition is directly responsible for yield reduction [9].

In agreement with this finding, similar results were reported as FHB has significant effect on kernel number per spike, grain yield and FKD in different parts of the world. Many authors reported as the disease has negative effect on yield (Osborne and Stein, 2007), grain quality, seed quality. Mesterhazy also stated that measuring yield reduction due to the disease along with visual symptoms of spike infections and the levels of FDK are used to determine the resistant level of cultivars. This showed that the disease causes a significant reduction in wheat grain quality and quantity by affecting directly kernel development resulted in floret abortion or development of small, shriveled and lightweight kernels that may be lost during harvest and cleaning operations [10].

Correlation Analysis of FHB Intensity and Yield Components

In this study, when the relationship between the disease parameters (DI, PSI & AUDPC) and yield components were considered significant correlations were observed based on the screening results from 38 Ethiopian wheat varieties. There were significantly positive correlations between FHB severity and FDK ($r=0.6$; $p < 0.01$), FHB severity and Yield loss ($r=0.63$; $p < 0.01$), AUDPC and FDK ($r = 0.55$; $p < 0.01$) & AUDPC and yield loss ($r=0.69$; $p<0.01$) as summarized. Fusarium head blight (FHB) incidence also had significantly positive correlation with both FDK($r=0.68$) and yield loss (0.67). Strong positive correlation also made between FDK and FHB disease incidence ($r=0.68$; $p \leq 0.05$), severity ($r=0.6$; $p \leq 0.05$), AUDPC ($r=0.55$; $p \leq 0.05$), PSI ($r=0.54$; $p \leq 0.05$), yield loss ($r=0.45$; $p \leq 0.05$). This suggested that the higher disease intensity, have positive correlation with yield and yield components and other quality parameters of the production. Muthomi reported as these parameters have directly relationship and they are used for varietal evaluation for the disease resistance. Similarly, found significant correlations among FHB, Fusarium-damaged kernels and yield loss.

Conclusion and Recommendations

The result of the study showed majority of the evaluated Ethiopian wheat varieties exhibited susceptible reaction to FHB and the disease becoming an important wheat production constraint in in the country. From the evaluated varieties, 31(81.6%) varieties exhibited susceptible reaction and only 7 (18.4%) varieties, namely Enkoy, Huluka, Galema, Hogana, K6295-4A, Dashen and Derselegne showed moderately tolerant reaction to the disease. All of the identified varieties as moderately tolerant to the disease were from bread wheat types. However, all durum wheat types used in this study showed susceptible reaction to the disease. This is might be due to the limited genotypes and sources of resistances of this wheat types. Majority of the currently cultivated wheat varieties in study area are among the susceptible and highly susceptible varieties identified in this study. This indicated (Figure 4) wheat production in Ethiopia has been seriously threatened by FHB epidemics. The epidemics and distribution of the disease might be increased with time since there is no any known immune/resistant cultivar against the disease in the country. These conditions may make as the disease remains as threat to crop production in the country. The highest yield loss (58.20%) by the disease also noted on the susceptible and dominant wheat variety (Ogolchu) in the area. The significant positive correlation ($p \leq 0.05$) among FHB intensity with yield and yield components were known by the study. The identified moderately tolerant varieties by this study should be combined with other proper agronomic practices and application of efficient fungicides at proper time could be recommended as the frontline to reduce the disease problem. Massive screening of introduced and locally available wheat genotypes including those evaluated in this study over years and locations to develop better resistant cultivars for use in wheat improvement program is also needed.

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