MYCOBIOLOGY
2018, VOL. 46, NO. 3, 283–286
https://doi.org/10.1080/12298093.2018.1496637

RESEARCH NOTE

OPEN ACCESS

Timing of *Fusarium* Head Blight Infection in Rice by Heading Stage

Yangseon Kim*, In Jeong Kang, Dong Bum Shin, Jae Hwan Roh, Sunggi Heu and Hyeong Kwon Shim

Crop Cultivation and Environment Research Division, National Institute of Crop Science, Rural Development Administration, Suwon, Korea

ABSTRACT

Fusarium graminearum causes the devastating plant disease *Fusarium* head blight and produces mycotoxins on small cultivated grains. To investigate the timeframe of *F. graminearum* infection during rice cultivation, a spore suspension of *F. graminearum* was applied to the rice cultivars Dongjin 1 and Nampyeongbyeo before and after the heading stage. The disease incidence rate was the highest (50%) directly after heading, when the greatest number of flowers were present, while only 10% of the rice infected 30 days after heading showed symptoms. To understand the mechanism of infection, an *F. graminearum* strain expressing green fluorescent protein (GFP) was inoculated, and the resulting infections were visually examined. Spores were found in all areas between the glume and inner seed, with the largest amount of GFP detected in the aleurone layer. When the inner part of the rice seed was infected, the pathogen was mainly observed in the embryo. These results suggest that *F. graminearum* migrates from the anthers to the ovaries and into the seeds during the flowering stage of rice. This study will contribute to uncovering the infection process of this pathogen in rice.

ARTICLE HISTORY
Received 14 June 2017
Revised 25 July 2017
Accepted 15 June 2018

KEYWORDS
Fusarium head blight; *Fusarium graminearum*; infection; rice

Fusarium head blight (FHB) affects cultivated grains such as rice, wheat, and barley. This infection is caused by the pathogen *Fusarium graminearum* [1], which is found all over the world, including Brazil, China, India, Japan, and Nepal [2]. In southern Korea, a severe epidemic of FHB in rice caused by *F. graminearum* occurred in 2001 after a heavy rainfall during the rice flowering period [3]. It has been reported that *F. graminearum* lineage 6 dominates in the warmer southern regions of the Korean peninsula where barley is grown, which is consistent with the previously reported dominance of *F. graminearum* lineage 6 in warmer climates [3].

The occurrence of *Aspergillus* and *Penicillium* spp. was reported in rice processing complexes in 2005–2006 [4], and *Aspergillus*, *Penicillium*, and *Fusarium* spp. have been found in the by-products of rice processing [5]. In addition, *Aspergillus*, *Penicillium*, and *Fusarium* infections were detected in rice kept in a rice processing complex in 2009 [6]. Thus, most reports of these fungal infections have pertained to cereals during storage for distribution. There has been little research into the timing and mode of FHB infection during rice cultivation.

Members of the *F. graminearum* species complex are known to cause diseases such as head blight, scab, and ear blight in barley, rice, maize, cucumber, potato, bean, and various cereal crops [7–10]. These fungi produce mycotoxins such as deoxynivalenol, nivalenol, and zearalenone [11–14]. These toxins are known to cause digestive disorders and to lower immunity and fertility when consumed by humans or livestock [14].

Recently, the occurrence of FHB has been increasing in rice fields before and after heading due to increased temperatures and rainfall [3]. FHB-infected ears of rice become irregular in shape and lightweight. Later in the infection, the infected ears change color to yellow, salmon, or brown, and in severe cases, the whole grain turns brown (Figure 1). Until recently, the occurrence of FHB in rice was reported mainly from areas of former barley and wheat cultivation [15]. However, in recent years, rice FHB has been found in areas of rice single cropping. Thus, research on the physiology and ecology of rice FHB, as well as barley and wheat FHB, is essential for the development of methods to control this disease. Research on FHB occurrence in barley has been conducted in the past, but rice FHB has rarely been documented. However, when a survey of the occurrence of FHB in rice fields was conducted by the National Institute of Crop Science in an area where barley FHB disease had occurred in

CONTACT Hyeong Kwon Shim shimhk@korea.kr
*Current address: Center for Industrialization of Agricultural and Livestock Microorganisms, Jeongeup-si, 56212, Korea.

© 2018 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group on behalf of the Korean Society of Mycology.
This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Figure 1. Symptoms of FHB on rice after artificial inoculation with *F. graminearum* isolate Z39G418 expressing GFP in the z3639 background. Spore suspension was adjusted to 10^4 spores/mL.

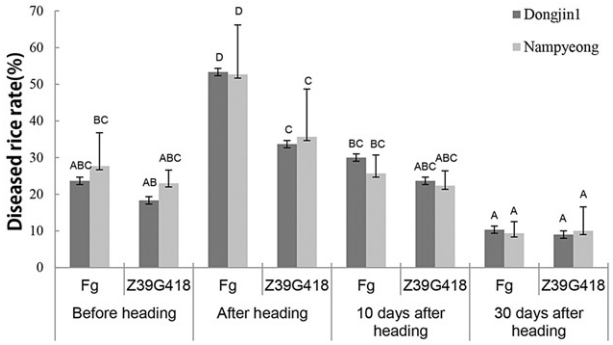

Figure 2. Comparison of pathogen detection rates between the *F. graminearum* wild-type strain z3639 and the GFP-expressing strain Z39G418. The strains were tested on two rice cultivars (Dongjin 1 and Nampyeongbyeo) and inoculated at different rice developmental stages before and after heading, over three replicates. Duncan's test was used to determine significance at the 95% probability level. The same letters indicate no significant difference between results.

2005, 25.8% of the study area was found to be infested with FHB.

In wheat, susceptibility to FHB is thought to be highest during the heading period, but such details of the infection method have not yet been experimentally determined in rice [16]. To investigate whether rice plants are also most susceptible to FHB infections during the heading stage, we examined the disease severity that resulted when inoculating pathogens before and after the rice heading period. The stages chosen reflect the fact that the flowering and grain-filling stages of rice begin within one to five days after heading and that grain filling is complete within three weeks. Additionally, to identify the infection sites in rice seeds, *F. graminearum* strain Z39G418, which expresses green fluorescent protein (GFP) in the wild-type background (z3639), was used for inoculation [17].

The cultivars used in this experiment were Dongjin 1 and Nampyeongbyeo, which are heavily affected by FHB. The plants were cultivated in a greenhouse from seed sterilization until shortly before the heading stage; the plants were then inoculated with FHB pathogens. Inoculation was performed with a 1×10^5/mL spore suspension of *F. graminearum* strains z3639 and Z39G418 at four developmental stages: before heading, immediately after heading, 10 days after heading (milky stage), and 30 days after heading (mature stage). After inoculation, the plants were covered with polypropylene bags for 2 days as a wet treatment. Then, the disease incidence was checked after 7 days (Figure 2). Spores of the FHB pathogen *F. graminearum* in the wild are carried to plant spikes by wind or splashing water, and they enter plants mostly through the flowers. Wheat, barley, and other grass species are most susceptible to FHB infection starting at the flowering stage (anthesis) [18].

In addition, FHB pathogens are known to be transferred to the ovary via contaminated pollen, and then the disease can spread throughout the plant [19,20]. In the present study, an infection rate of at least 10% was found after inoculation in all stages, from the period before heading to 30 days after heading, regardless of the rice cultivar. The disease incidence rate was the highest after heading, at approximately 50% (Figure 2). Our data are highly similar to the results of Kang et al. [16]. This high disease rate can be attributed to the fact that more than 80% of the flowers will bloom at this stage and that most of the pollen is exposed. In the case of rice inoculated 10 days after heading (milky stage), the pathogen detection rate was 23–30% (Figure 2). Even when the plants were inoculated 30 days after heading, the detection rate was approximately 9–10%, though almost all the flowers were withered and lacking pollen. The pathogen appeared to infect the glumes and spread from there into the host plants. FHB infection was most effective during the peak flowering time of rice, but infection was still possible well after flowering.

Recent studies have shown that the anthers and ovaries are the primary sites of infection for *F. graminearum*. In addition, the adaxial sides of

glumes are accessible infection sites where spores can attach and spread further [20]. To investigate whether the FHB pathogen can proliferate in rice and to determine the susceptibilities of different rice growth stages, an *F. graminearum* strain expressing GFP in the wild-type background was used for further inoculation.

The GFP-expressing strain was inoculated at different stages of rice development, and there was no significant difference in the disease detection rate between different stages, except for the period just after heading (Figure 2). Although the GFP-expressing strain showed a lower infection rate after the heading stage compared to the wild type, GFP was instrumental in discovering the infection sites in rice. Infection sites were studied by inoculation of the rice cultivars Dongjin 1 and Nampyeongbyeo at the following stages: before heading, directly after heading, and 10 days after heading.

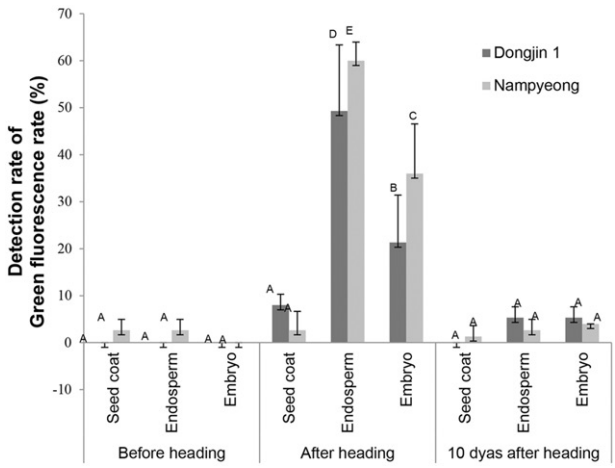

Figure 3. Detection rates of the FHB pathogen in seeds following inoculation with the GFP-expressing strain Z39G418 at different rice developmental stages before and after heading, over three replicates. Duncan's test was used to determine significance at the 95% probability level. The same letters indicate no significant difference between results.

The diseased ears were collected and fixed with formaldehyde–acetic acid–ethanol (FAA). Paraffin sections were made with a microtome (Shandon MT-960; Thermo Shandon Inc., Pittsburgh, PA, USA). GFP localization was examined by fluorescence microscopy (Leica DMRE; Leica Microsystems, Wetzler, Germany) and confocal microscopy (Leica MZ16A; Leica Microsystems, Wetzler, Germany). Inoculated seeds were dissected to separate the seed coat, endosperm and embryo, and then the GFP-expressing pathogen was surveyed (Figure 3).

The largest amount of fungal material was detected in the endosperm (30%) when the pathogen was inoculated after heading or 10 days after heading. This suggests that the pathogens could penetrate the gaps between the inner and outer parts of the glumes, resulting in the growth of pathogens in the endosperm. GFP-expressing pathogens were detected at a rate of approximately 10%

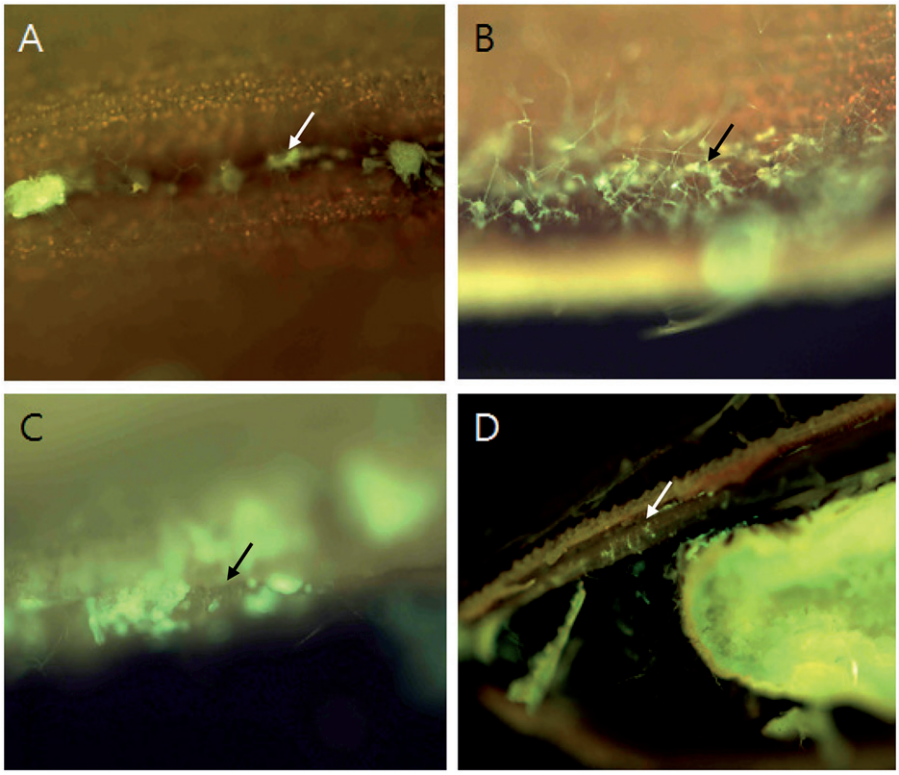

Figure 4. Microscopy images of the interior of infected rice seeds inoculated with the GFP-expressing *F. graminearum* strain Z39G418 were captured with the Leica TCS SP2 microscope. (A) inner glume, (B) inner seed coat, (C) surface of endosperm cells, (D) embryo bud. Arrows indicate the presence of the pathogen.

in the seed coat. Before heading, even if a large inoculum of the FHB pathogen was present in the air, spores could not settle on anthers to be transmitted to the seeds. However, when the number of exposed flowers was the highest, after heading, the rates of seed colonization and GFP detection were also high. Many flowers had disappeared by 30 days after heading and the number of pathogens invading seeds also decreased. In other words, seed colonization correlated with the number of open flowers. Next, seeds showing symptoms of FHB were cut using a microtome to examine the pathogen infection sites under a fluorescence microscope, and FHB pathogens were detected in several parts of the seed (Figure 4). In rice, the FHB fungus proliferated mainly between the seed coat and the endosperm, and spores formed in the seed coat (Figure 4). Together, these results suggest that rice FHB pathogens initially infect the anthers then spread to the ovaries via the pollen and proliferate in the seeds, as is the case for barley. Our analysis will contribute to uncovering the infection process of the FHB pathogen in rice.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This study was supported by Cooperative Research Program for Agriculture Science & Technology Development, funded by Rural Development Administration (PJ01016402), Republic of Korea.

References

[1] Leslie JF, Summerell BA. The Fusarium laboratory manual Ames, (IA): Blackwell Professional; 2006.

[2] Desjardins AE, Busman M, Manandhar G, et al. *Gibberella* ear rot of maize (*Zea mays*) in Nepal: distribution of the mycotoxins nivalenol and deoxynivalenol in naturally and experimentally infected maize. J Agric Food Chem. 2008;56: 5428–5436.

[3] Lee J, Chang I-Y, Kim H, et al. Genetic diversity and fitness of *Fusarium graminearum* populations from rice in Korea. Appl Environ Microbiol. 2009;75:3289–3295.

[4] Oh JY, Lee SN, Nam Y, et al. Populations of fungi and bacteria associated with samples of stored rice in Korea. Mycobiology. 2007;35:36–38.

[5] Lee T, Lee SH, Lee SH, et al. Occurrence of *Fusarium* mycotoxins in rice and its milling by-products in Korea. J Food Prot. 2011;74:1169–1174.

[6] Son SW, Nam YJ, Lee SH, et al. Toxigenic fungal contaminants in the 2009-harvested rice and its milling by products samples collected from rice processing complexes in Korea. Res Plant Dis. 2011;17:280–287.

[7] Bily AC, Reid LM, Savard ME, et al. Analysis of *Fusarium graminearum* mycotoxins in different biological matrices by LC/MS. Mycopathologia. 2004;157:117–126.

[6] Goswami RS, Kistler HC. Heading for disaster: *Fusarium graminearum* on cereal crops. Mol Plant Pathol. 2004;5:515–525.

[8] Ali S, Rivera VV, Secor GA. First report of *Fusarium graminearum* causing dry rot of potato in North Dakota. Plant Dis. 2005;89:105.

[9] Pioli RN, Mozzoni L, Morandi EN. First report of pathogenic association between *Fusarium graminearum* and soybean. Plant Dis. 2004;88:220.

[10] Ferrigo D, Raiola A, Causin R. *Fusaium* toxins in cereals: occurrence, legislation, factors promoting the appearance and their management. Molecules. 2016;21:627–662.

[11] Jimenez M, Mateo R. Determination of mycotoxins produced by *Fusarium* isolates from banana fruits by capillary gas chromatography and high-performance liquid chromatography. J Chromatogr A. 1997;778:363–372.

[12] Lee SH, Son SW, Nam YJ, et al. Natural occurrence of *Fusarium* mycotoxins in field-collected maize and rice in Korea in 2009. Res Plant Dis. 2010;16:306–311.

[13] Lee T, Lee S, Kim L, et al. Occurrence of fungi and *Fusarium* mycotoxin in the rice samples from rice processing complex. Res Plant Dis. 2014;20: 289–294.

[14] Lee W, Lee H, Ki K, et al. Effects of barley and barley bran contaminated with *Fusarium* spp. on the growth and feed efficiency of fattening and growing pigs. Korean J Vet Res. 2012;52:45–52.

[15] Ryu JG, Lee S, Lee S, et al. Natural occurrence of *Fusarium* head blight and its mycotoxins in 2010-harvested barley and wheat grains in Korea. Res Plant Dis. 2011;17:272–279.

[16] Kang Z, Zingen-Sell I, Buchenauer H. Infection of wheat spikes by *Fusaium avenaceum* and alterations of cell wall components in the infected tissue. Eur J Plant Pathol. 2005;111:19–28.

[17] Son H, Lee J, Lee Y-W. A novel gene, *GEA1*, is required for ascus cell-wall development in the ascomycete fungus *Fusarium graminearum*. Microbiology (Reading, Engl).). 2013;159: 1077–1085.

[18] McMullen M, Bergstrom G, De Wolf E, et al. A unified effort to fight an enemy of wheat and barley: *Fusarium* head blight. Plant Dis. 2012;96: 1712–1728.

[19] Han O, Kim J. Establishment of artificial screening methods and evaluation of barley germplasms for resistance to *Fusarium* head blight. Korean J Crop Sci. 2005;50:191–196.

[20] Rittenour WR, Harris SD. An *in vitro* method for the analysis of infection-related morphogenesis in *Fusarium graminearum*. Mol Plant Pathol. 2010;11:361–369.