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Evaluation of deoxynivalenol and virulence in dsRNA containing Fusarium graminearum Isolates

T. Pavan
Narendra Deva University of Agricultural Technology

Abstract.
Hypovirulence and the presence of double-stranded RNA (dsRNA) viruses in some fungus have led to their potential use as biological control agents. This study screened three dsRNA-containing and one dsRNA-free strain of Fusarium graminearum, the causative agent of wheat head blight, to assess the influence of dsRNA on pathogenicity and deoxynivalenol (DON) synthesis. In a greenhouse experiment, sensitive wheat (cv. Falat) was seen to have a considerably lower disease severity ($p<0.01$) when exposed to the dsRNA-containing isolates compared to the dsRNA-free isolates. HPLC methods validated DON synthesis by F. graminearum isolates with and without dsRNA. A range of 0.07 to 1.62 ppm and 0.06 to 0.4 ppm was observed for in vitro DON formation, respectively. In comparison to the dsRNA-free isolates, dsRNA-containing derivatives showed a 50% reduction in DON. In the meanwhile, spikes infected with F. graminearum isolates containing dsRNA ranged from 0.37 to 0.63 ppm, whereas spikes injected with dsRNA-free varied from 0.56 to 0.9 ppm. According to these findings, the generation of DON was reduced by 27.5%.

Keyword Fusarium graminearum, Deoxynivalenol, dsRNA, hypovirulence

Introduction

Fusarium head blight (FHB) is a terrible disease that causes significant losses in wheat production and quality in humid and semi-humid parts of the globe. It is caused by Fusarium graminearum Schwabe, a teleomorph of Gibberella zeae (Schwein) Petch. (McMullen et al., 1997; Champeil et al., 2004). Toxins such the trichothecene deoxynivalenol (DON) may be produced by F. graminearum in addition to FHB, which lowers wheat grain quality. Animal and human health are greatly affected by the presence of DON in wheat grains. According to Snidjers (1990) and the World Health Organization (2001), trichothecenes may cause skin poisoning, toxic aleukia, nausea, vomiting, and impaired immunological systems. According to Clear and Patrick (2000), DON is found in the greatest quantities and most often discovered compounds globally.
cited as potential biological pesticides in a 2002 study by Chu et al.
According to the first study on double-stranded RNA infection in Fusarium graminearum isolates, the morphological changes brought about by the dsRNA include a decrease in mycelial development, an increase in pigmentation, a decrease in virulence towards wheat, and a sixty-fold reduction in the generation of Dionaemine mycotoxin (DON). According to Chu et al. (2002), alterations in pathogenicity and morphology were associated with the presence or lack of dsRNA. According to research conducted by Hashemi et al. (2004) and Aminian et al. (unpublished data), screening of F. graminearum isolates in Iran revealed that 7% of the isolates were viruliferous and included fragments ranging from 1.5 to 6 kb. In this investigation, we aimed to identify how dsRNA infection affected the DON production of F. graminearum isolates in both laboratory and greenhouse settings.

Ghabrial (1998) and Varga et al. (2003) identified a large diversity of fungi and yeasts that harbor double-stranded RNA (dsRNA) mycoviruses. While infected, mycoviruses may live for a long time and often cause no symptoms at all. But dsRNA mycovirus infection may occur in some types of fungus.1. Sporadic fungi

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triggers unique physiological and morphological alterations, includingThe pigmentation and enzymatic activities were studied using F. graminearum isolates (F38, F42, and F118) originally isolated from wheat in Ardebil and Golestan provinces, Iran. The isolates were compared with three dsRNA-containing and three dsRNA-free strains, as well as with strains originally isolated from wheat in terms of virulence-associated traits like growth rate and sporulation. Mycotoxins produced by mycoviurses, with or without dsRNAs, consistently reduce the expression of novel virulence-related traits, such as hypovirulence (Newhouse, unpublished data), hypovirulence (Magliani et al., 1997; Varga et al., 1994), and F. graminearum (et al., 2004; Aminian et al., 2004). Lauren and Agnew's (1991) approach for in vitro DON analysis. Is the rice edible?2. Two plugs were taken from the margin of 4-well plates to inoculate the spike culture medium with DON. The DON analysis followed the same steps as described earlier, with the exception that the spikes of each treatment were agar instead of potato dextrose. The cultures were then incubated at 25 ºC for 5 weeks, mixed, and finally ground with liquid nitrogen. Only 2.2 grams of a 3 g subsample Procedure and cleanup: a mixture of acetonitrile, methanol, and water (16:1, 3:1) was used for extraction. A temperature of 50 ºC was used to dry the mycelial mass and substrate. After 15 hours of shaking at 170 rpm in a 250 ml Erlenmeyer flask, the dry substrate weighed in grams was crushed in a blender extractor and filtered using Whatman No. 1 filter paper. Lauren and Agnew (1991) and Jening et al. (2004) both reported a 30-centimeter glass tube that was plugged with glass wool and dry-packed with For the purpose of creating a mini-clean up column, 60 ml of alumina/carbon (20:1 ; 1g) and 2g of prewashed cation exchange acetonitrile: methanol: water (16:1:3) were added to a 250 ml Erlenmeyer flask together with each grinding sample (15g). for three hours at 170 rpm in a rotary shaker, and then filtered the liquid.After applying the whole extract to the column, it was allowed to drain through Whatman no. 1 filter paper (Maidston, Kent, England). A as a result of gravity, and the effluent was collected. A mini-clean up column was created by passing the first column eluent through a dry-packed mini-column of alumina/carbon (20:1:1; 1g) and 2g of prewashed packing glass into a 30-cm+1cm ID glass tube with a small plug of glass wool and cation exchange resin. This completed the further cleaning process. The alumina/carbon mixture was put into a 1:1:1 ratio and then let to drain naturally. After adding the second filtrate to the column, we let it drain naturally. The eluent from the column was then evaporated at 70 °C until it was completely dry. Eluant was obtained along with residues. After 10 milliliters of the first column's elute had rested for 1 hour in a mixture of 10 parts methanol and 90 parts water, it was passed through a micro column constructed from a packed glass tube and left at room temperature for further cleanup. The remaining materials were then dissolved in a mixture of methanol (30 cm + 1 cm id), alumina/carbon (1:1), water (5:95; 2 ml), and acetonitrile-methanol (3:1), and the mixture was evaporated at 1 g. After that, gravity was let the elute drain. Second, 70 degrees Celsius. The remaining residues were dissolved in a mixture of 5 parts methanol and 95 parts water. The column elute was then dried by evaporation at 70 ºC. Until analysis, the residues were refrigerated at 4 °C. Each treatment's setup, incubation, and extraction took place at room temperature, and three duplicates were dissolved in a mixture of methanol and water (10:90; 2 ml). The trials were then performed twice using the dissolved residues.

much lower concentrations in dsRNA-containing compounds compared to their dsRNA-free analogues. The percentage of DON that was reduced varied between 5.4% and 77.1%. Potentially harmful effects

All wheat plants that were infected with either the dsRNA-free or dsRNA-containing F. graminearum isolates showed signs of head blight. Isolates that included dsRNA, on the other hand, showed much milder illness symptoms than those that did not. After being injected with ten microliter solutions of 104 conidia per ml, the disease severity levels of dsRNA-containing isolates ranged from 27% to 43%, whereas those of dsRNA-free isolates ranged from 69% to 81%. Figure 1 shows that the control

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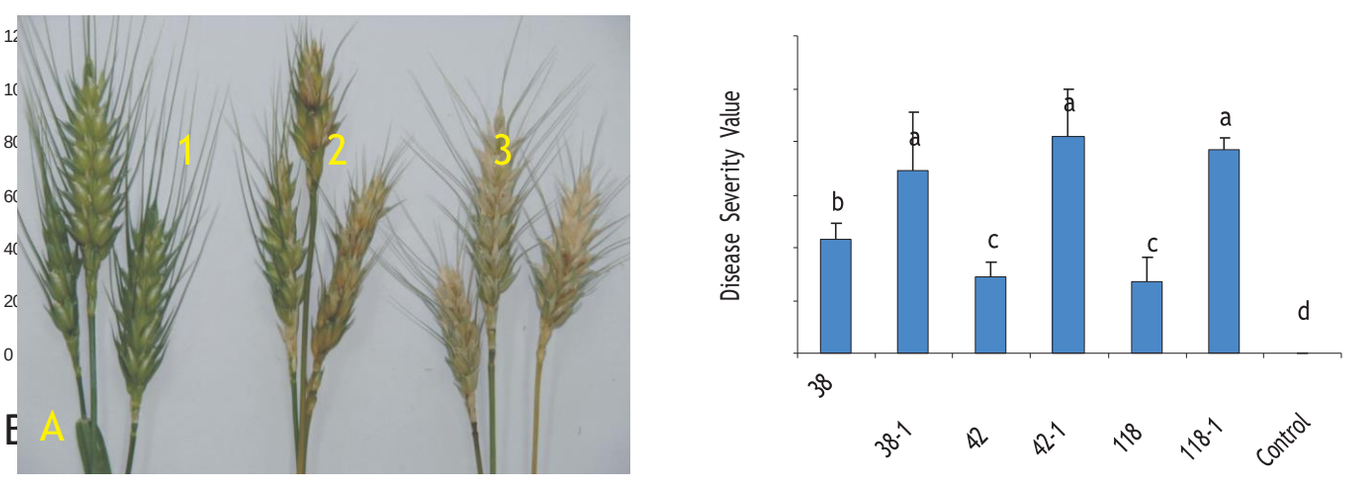
inoculation group did not experience any side effects. an increase in DON output Table 2 shows the quantity of DON generated in spikes infected with F. graminearum isolates that include and those without dsRNA. Isolates containing dsRNA produced DON at relative levels of 0.37 to 0.63 ppm, whereas those without dsRNA produced DON at values ranging from 0.56 to 0.9 ppm. In comparison to the dsRNA-free equivalent isolates, DON was found at much lower concentrations in the dsRNA-containing derivatives. Table 2 shows that the percentage of DON that was reduced varied between 21.7% and 33.9%.

For three days, the plants were kept in a plastic bag at a high relative humidity. After 10 days, the wheat heads that had been inoculated were examined in order to quantify the degree of FHB illness. Two separate runs of the experiment were conducted. Research on the impact of viral infection on toxin production used Duncan's studies and a multiple range test, which resulted in means being separated at $p<0.01$.a variety of patterns have been generated by certain types of fungi. Still, a



solates

Figure 1. Pathogenicity test and Disease severity

(A) In order to assess the pathogenicity of the wheat plants, conidial suspensions were injected onto the plants. The symptoms were more severe in plants infected with F-118 (3) spores that did not include dsRNA compared to plants infected with F-118 (2) spores that did contain dsRNA. First, control plants were injected with water. Second, F. graminearum isolates that included dsRNA were compared to those that did not, in terms of disease severity. When the letters are different, it means that there are 1% statistical variations between the isolates. relationship between the pathogenicity of the wheat spike in a greenhouse and its toxin production. Additionally, according to Bozarth et al., dsRNA-free isolates of the maydis, the causative agent of southern corn blight, were much more severely diseased than dsRNA-containing isolates of Helminthosporium. These findings are consistent with those that were published in 1972. Investigating the root-and-chu et al. (2002)-causing Periconia circinata. They demonstrated that dsRNA inhibits sorghum corn rot, but they didn't find any correlation between virulence towards wheat or a 60-fold drop in toxin production depending on whether or not viral particles trichothecenes mycotoxin (DON) were

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present. Dunkle (1974) found a correlation between changes in pathogenicity and the presence or lack of dsRNA.
We used a rice substrate to examine DON morphogenesis in vitro (Chu et al., 2002). research. The majority of mycotoxins generated by the fungus Fusarium includeSince trichothecene synthesis is involved in virulence, dsRNA carrying F. graminearum isolates exhibited much slower growth when grown on solid substrates like as grains (often rice and maize). Infected wheat plants must undergo disease development and extraction before these cultures may be used. Further chromatography, gas liquid chromatography, or high performance investigations are necessary to validate these findings since the development of the purification process before identification by thin layer illness is a complex event.
using liquid chromatography (Geraldo et al., 2006).Reduced grain output due to head blight and often Our findings indicate that mycotoxin (DON) production is significantly reduced in F. graminearum isolates that carry dsRNA contaminated with trichothecenes compared to those that do not (Marasas et al., 1984). Lower crop yields and poor grain quality are two examples of the direct economic losses that may occur as a consequence of the infection; these have become commonplace in both in vitro rice cultures and in the real world (KE, 1986). Assessment of several deoxinivalenol sources for feed (Windels, 2000; Chamley et al., 1994). Swine may also be fed a substance that causes vomiting. Fears over the effects of exposure to 1149-1154 on public health were voiced in the Canadian Journal of Animal Science, volume 66.
Food intolerance, nausea, and skin lesions caused by fusarium mycotoxins In 1998, Ghabrial SA reported... Necrosis: its history, adaptations, and potential evolutionary routes (Chamley et al., 1994; Foster et al., 1986). To what extent do dsRNA fungal viruses spread? Article published in Virus Genes, volume 16, pages 119–131.
dsRNA-free cells may receive the F-38, F-42, and F-118 fragments Isolates by hyphal fusion with a high occurrence rate, as noted by Hashemi, Mozafari, Alizadeh, and Shams-bakhsh in 2004. Iran decreases the virulence level and mycotoxin generation of dsRNAs linked to Fusarium graminearum isolates. The citation is from the Iranian Journal of Plant Pathology, volume forty, pages 351-326.
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