Variation in 8-ketotrichothecenes and zearalenone production by *Fusarium graminearum* isolates from corn and barley in Korea

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(Received: 16 January 1996; Accepted in final form: 20 February 1996)

Abstract

A total of 214 *Fusarium graminearum* isolates were obtained from corn and barley which were collected from Kangwon province and the southern part of Korea, respectively, and were tested for 8-ketotrichothecenes and zearalenone (ZEA) production on rice grains. The incidences of trichothecene production by 105 isolates of *F. graminearum* from corn were 59.0% for deoxynivalenol (DON), 37.1% for 15-acetyldeoxynivalenol (15-ADON), 13.3% for 3-acetyldeoxynivalenol (3-ADON), 7.6% for 3,15-diacetyldeoxynivalenol (3,15-DADON), 20.0% for nivalenol (NIV), 6.7% for 4-acetylnivalenol (4-ANIV), and 1.0% for 4,15-diacetylnivalenol (4,15-DANIV). DON chemotypes frequently produced 15-ADON as the major isomer rather than 3-ADON and 9 of the 61 DON chemotypes produced low levels of NIV. On the other hand, the incidences of trichothecene production of 109 isolates by *F graminearum* from barley were 24.8% for DON, 72.5% for NIV, 62.4% for 4-ANIV, and 10.1% for 4,15-DANIV. Of these isolates, 78 were NIV chemotypes and only one isolate produced DON and 3-ADON as major toxins. In addition, 26 of the 78 NIV chemotypes produced low levels of DON. ZEA was frequently produced by the trichothecene-producing isolates and the incidences of ZEA were 51.4% and 31.2% for the isolates from corn and barley, respectively. There was a great regional difference in trichothecene production by *F. graminearum* isolates between corn- and barley-producing areas in Korea.

Key words: barley, chemotaxonomy, corn, *Fusarium graminearum*, 8-ketotrichothecenes, zearalenone

Introduction

*Fusarium graminearum* Schwabe (*Gibberella zeae* Petch), one of the major causative fungi of cereal scab, produces toxic metabolites including deoxynivalenol (DON), 15-acetyldeoxynivalenol (15-ADON), 3-acetyldeoxynivalenol (3-ADON), 3,15-diacetyldeoxynivalenol (3,15-DADON), nivalenol (NIV), 4-acetylnivalenol (4-ANIV), and 4,15-diacetylnivalenol (4,15-DANIV) as well as an estrogenic mycotoxin, zearalenone (ZEA). The structures of 8-ketotrichothecenes are shown in Figure 1. Among these, DON and NIV have been frequently found in cereals harvested in oriental countries [1–8]. Contamination of cereals with NIV is of great concern because the acute toxicity of NIV is definitely greater than that of DON [9].

Based on the production of 8-ketotrichothecenes, Ichinoe et al. [10] reported that *G. zeae* is chemotaxonomically divided into two groups; one is the NIV chemotype which produces NIV and 4-ANIV, and the other is the DON chemotype which produces DON and 3-ADON. ZEA is often produced by these two chemotypes. Lee et al. [11] and Logrieco et al. [12] also reported the two chemotypes in *Fusarium* isolates from cereals in Korea and Italy, respectively. It appears that there are geographical differences in the natural distribution of the two chemotypes. For example, the presence of the NIV chemotype was reported in Korea, Japan, Taiwan, and Italy [10–14]; however, the NIV chemotype was not reported in North American countries such as Canada and the United States, although grain contamination with NIV was reported in Canada [15, 16]. North American isolates of *F. graminearum* produce DON and 15-ADON as the major toxins [17].
In a previous paper [1], we reported that the pattern of the natural occurrence of monoacetyl-DON and the ratios of NIV to DON in Korean barley and corn were different for each cereal. The purpose of this study was to evaluate the mycotoxins produced by 214 isolates of *F. graminearum* from barley and corn samples for the production of 8-ketotrichothecenes and ZEA and to determine the variation in trichothecene production by *F. graminearum* isolates in Korea.

**Materials and methods**

**Corn and barley samples**

A total of 39 cereal samples were collected. Fifteen corn samples were collected from different farmers’ stocks in Kangwon province during November in 1992. Twenty-four barley samples were collected from Chonbuk, Chonnam, Kyungbuk, and Kyungnam provinces during July 1993.

**Isolation of Fusarium species**

From each grain sample, 100 kernels were randomly selected, shaken in 2% NaOCl for 1 min, rinsed in sterile distilled water, and transferred to potato dextrose agar plates followed by incubation at 25 °C for 4–7 days. *Fusarium* species were transferred from grains to noncommercial potato dextrose agar or carnation leaf agar [18], or both, incubated under fluorescent lamps (5,000 lux) at 25 °C, and identified using the method of Nelson et al. [19]. Because the *Fusarium* colonies isolated from two cereal samples were numbered into the hundreds, only *F. graminearum* isolates (214 isolates) were selected and assayed for mycotoxins. Stock cultures of *F. graminearum* isolates were single spore isolated. They were maintained on autoclaved soil media and stored at −15 °C.

**Chemicals**

Trichothecene mycotoxins, including DON, 15-ADON, 3-ADON, 3,15-DADON, NIV, 4-ANIV, 4,15-DANIV, and ZEA were kindly supplied by Dr. T. Yoshizawa, Department of Bioresource Science, Faculty of Agriculture, Kagawa University, Japan. Each mycotoxin was dissolved in methanol at a concentration of 1 mg/ml and stored at 4 °C. A trimethylsilylating reagent was prepared with a N-trimethylsilyl-imidazole-N,O-bis(trimethylsilyl)acetamide-trimethylchlorosilane at a ratio of 3 : 3 : 2 (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

**Preparation of cultures**

Erlenmeyer flasks (500 ml), each containing 100 g of rice and 60 ml of distilled water, were autoclaved twice for 1 hr at 121 °C with a 24-hour interval. The rice was inoculated with mycelium plugs from 5-day-old potato dextrose agar of each isolate. The flasks were incubated for 4 weeks at 25 °C. The mycelial mass and substrate were disbursed onto a screen-bottom tray and allowed to air dry in a ventilated hood. When dry, this inoculated substrate was ground to the consistency of flour and stored at −15 °C until analysis.

**Extraction and clean up**

The rice cultures were extracted by the procedure reported previously [20]. Each ground culture (20 g) was extracted with 160 ml of acetonitrile-water (3 : 1, vol/vol) for 30 min and the extract was filtered through Whatman No. 1 filter paper. An 80-ml filtrate was defatted with the same volume of n-hexane and concentrated to dryness. The residue was dissolved in 2 ml of methanol and applied onto a Florisil column (2 cm [inside diameter] by 15 cm) containing 10 g of Florisil (60/100 mesh, Fisher Scientific Co., Pittsburgh, PA, USA). The column was washed with 100 ml of n-hexane, followed by elution with 100 ml of