Natural Occurrence of *Fusarium* Mycotoxins (Trichothecenes and Zearalenone) in Barley and Corn in Korea

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Barley is produced in four provinces, Chonbuk, Chonnam, Kyungbuk, and Kyungnam, and corn is mainly produced in the Kangwon province in Korea. The natural occurrence of *Fusarium* mycotoxins was surveyed in 39 barley and 46 corn samples from different areas. Five 8-ketotrichothecenes, namely deoxynivalenol (DON), nivalenol (NIV), 4-acetyldeoxynivalenol (4-ANIV), 3-acetyldeoxynivalenol (3-ADON), and 4,15-diacyctylnivalenol (4,15-DANIV), and zearalenone (ZEA) were detected in barley. DON, NIV, and ZEA were the major contaminants in barley, with mean levels of 170, 1,011, and 287 ng/g, respectively. On the other hand, DON, 15-acetyldideoxynivalenol (15-ADON), NIV, 4-ANIV, 4,15-DANIV, and ZEA were detected in corn samples. DON and 15-ADON were the major contaminants in corn, with mean levels of 310 and 297 ng/g, respectively. The survey indicated that the natural occurrence of monoacetyl-DON and the ratios of NIV to DON in two cereals were different. In addition, this is the first report of the natural occurrence of 4,15-DANIV in cereals.

Cereal scab is caused by species of *Fusarium* and sometimes results in toxic effects on humans and farm animals following consumption of *Fusarium*-infected cereals. *Fusarium graminearum* Schwabe (Gibberella zeae Petch), one of the major causative fungi of cereal scab, produces trichothecenes, such as deoxynivalenol (DON), 15-acetyldideoxynivalenol (15-ADON), 3-acetyldeoxynivalenol (3-ADON), nivalenol (NIV), and 4-acetyldeoxynivalenol (4-ANIV, fusarenon-X), as well as an estrogenic mycotoxin, zearalenone (ZEA). Among these, the mycotoxins often encountered in cereals are DON, NIV, and ZEA in oriental countries (9, 10, 15–19, 28, 30). Recently, Luo et al. (15, 16) reported the presence of 15-ADON and 3-ADON in corn and wheat, respectively.

Barley is an important crop in four provinces, Chonbuk, Chonnam, Kyungbuk, and Kyungnam, and corn is mainly produced in the southern part of Korea. Naked barley is a staple food, with rice and husked barley being used mainly for brewing. Barley is sown in the autumn and harvested during the early summer before other cereal crops. Historically, severe epidemics of cereal scab occurred in the southern part of Korea in 1963 (3, 4). The yield loss of cereals was 80 to 100% in some areas, causing a social problem because of not only heavy economic losses but also mycotoxicoses in humans and farm animals. Nausea and vomiting occurred within 5 to 15 min after consuming a meal containing scabby barley, and diarrhea, headache, dizziness, and throat irritation occurred within several hours to 24 h in humans (3). However, the toxic mechanisms remained unknown until some publications in the 1980s indicated that DON, NIV, and ZEA were major mycotoxins in Korean wheat and barley contaminated with *Fusarium* species (9–11, 18).

On the other hand, corn is mainly produced in the Kangwon province, which is located in the mideastern part of Korea. It is sown in the early summer and harvested during the autumn. During the growing period, corn stalk and ear rot is a serious problem in the Kangwon province. Although incidents of mycotoxicoses due to consumption of corn infected with *Fusarium* species have not been reported in this area, the natural occurrence of *Fusarium* mycotoxins in corn has been reported (17). Several investigators (8, 13, 14) have also reported that *Fusarium* isolates from corn in this area were highly toxic to experimental animals and could produce trichothecenes and ZEA.

For this report, we attempted to survey the natural occurrence of trichothecenes and ZEA in barley and corn samples from different areas of Korea.

**MATERIALS AND METHODS**

**Barley and corn samples.** A total of 85 cereal samples were collected. Thirty-nine barley samples were collected from different farmers' stocks in the Chonbuk, Chonnam, Kyungbuk, and Kyungnam provinces during July 1990. Corn kernels (46 samples) were collected from six counties in the Kangwon province during November 1990 and 1991. All grain samples were stored at −15°C until analysis.

**Chemicals.** Trichothecene mycotoxins, including DON, NIV, 15-ADON, 3-ADON, 4-ANIV, 3,15-diacyctylnivalenol (3,15-DADON), 4,15-diacyctylnivalenol (4,15-DANIV), and ZEA were prepared in T. Yoshizawa's laboratory. They were individually dissolved in methanol at a concentration of 1 mg/ml and stored at 4°C. A trimethylsilylating reagent was prepared with an N-trimethylsilylmida-zole-N,O-bis(trimethylsilyl)acetamide-trimethylchlorosilane at a ratio of 3:3:2 (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

**Extraction and clean up.** Barley and corn samples were extracted by using a slight modification of a previously published procedure (22). Each ground sample (40 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 sample of the 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.). The column was washed with 100 ml of n-hexane and then eluted with 100 ml of chloroform-methanol (9:1, vol/vol). The eluate was concentrated to dryness, and the residue was redissolved in 2 ml of methanol. For trichothecene analysis by gas chromatography-mass spectrometry (GC-MS), 1 ml of the sample solution was used, and the rest of the sample solution was used for ZEA analysis by high-performance liquid chromatography (HPLC).

**Quantitation and confirmation of trichothecenes.** A portion of each extract was reacted with the trimethylsilylating reagent and analyzed with a Shimadzu GCMS-QP 2000 gas chromatograph-mass spectrometer with a selected ion-monitoring (SIM) mode. The following equipment and conditions were used for the analysis: column, Shimadzu HiCap-CBPM50 fused silica capillary column (0.2 mm [inside diameter] by 50 m), 0.25-μm film (Shimadzu Scientific Instruments Inc., Kyoto, Japan) coated with methyl silicone (chemically bonded type); carrier, helium gas at a flow rate of 2 ml/min; column temperature, 5 min at 120°C and then increased to 280°C at 8°C/min; injector and interface temperature, 280°C; ion source temperature, 270°C; ionizing voltage, 70 eV; scanning rate, 1.5 scans/s; and sampling rate for SIM, 5 points/s. The m/zs for fragment ions monitored for quantitation of trichothecenes were 422 and 393 for DON, 377 and 287 for 3-ADON, 392 and 350 for 15-ADON, 379 and 289 for NIV, 480 and 450 for 4-ANIV, 320 for 3,15-DADON, and 408 for 4,15-DANIV. The calculation of trichothecene concentration was based on the average area counts of the fragment ions of each standard toxin. The retention time for each toxin was 25.22 min for DON, 26.43 min for 3-ADON, 26.54 min for 4-ANIV, 26.62 min for 15-ADON, 27.18 min for NIV, 27.89 min for 3,15-DADON, and 28.60 min for 4,15-DANIV. The detection limit of the method employed for trichothecene was 5 ng/g. When control barley and corn samples were spiked with 200 ng of each trichothecene per g of each sample, recoveries were 92 to 105% in barley and 87 to 95% in corn, respectively. All data were presented without the correction for recoveries.

In order to confirm the presence of 4,15-DANIV in corn, a corn sample (100 g) which was positive for 4,15-DANIV by GC-MS with SIM was extracted and purified as described above. The residue of the extract was chromatographed on a preparative thin-layer chromatography plate (Kieselgel 60, 20 μg 20 cm, 0.4-mm thickness; E. Merck, Darmstadt, Germany) with a 4,15-DANIV standard and developed in chlooroform-methanol (9:1, vol/vol). The region corresponding to 4,15-DANIV was scraped off and eluted with acetone, and the eluate was concentrated. The residue was derivatized with the trimethylsilylating reagent and analyzed by a JEOL AX 505 mass spectrometer equipped with an MSMP-DAP-2 data system. The equipment and analytical conditions used were as follows: column, DB-5 fused silica column (30 m by 0.25 mm [inside diameter]), 0.25-μm film; J&W Scientific, Folsom, Calif.; column temperature, 160°C for 5 min and then increased to 270°C at 5°C/min; injector temperature, 270°C; ion source temperature, 200°C; ionizing voltage, 70 eV; ionizing current, 300 μA; and scanning rate, 2 s/scan.

**HPLC analysis of ZEA.** A Shimadzu LC-6A HPLC equipped with a PF-110 spectrofluorometer (Japan Spectroscopic Co., Ltd., Tokyo, Japan) was used for the analysis of ZEA. For HPLC analysis, a Zorbax octadecylsilane column (4.6 mm [inside diameter] by 15 cm; particle size, 5 μm; Dupont Co., Kyoto, Japan) with a mobile phase of 70% aqueous methanol, a flow rate of 1 ml/min, an excitation wavelength of 236 nm, and an emission wavelength of 418 nm were used. The detection limit of the method for ZEA was 2 ng/g. When control barley and corn samples were spiked with 200 ng of ZEA per g of each sample, recoveries from barley and corn were 93 and 88%, respectively.

**RESULTS**

**Natural occurrence of trichothecenes and ZEA in barley.** The natural occurrence of 8-ketotrichothecenes (DON, 3-ADON, 4-ANIV, and 4,15-DANIV) and ZEA in barley samples is summarized in Table 1. Neither 15-ADON nor 3,15-DADON was detected in barley by GC-MS with the SIM mode. Figure 1B shows the chromatogram recorded by SIM of 3-ADON and 4-ANIV in one of the barley samples. The incidences of toxins in barley samples were 89.7% for DON, 17.9% for 3-ADON, 94.9% for NIV, 43.6% for 4-ANIV, 12.8% for 4,15-DANIV, and 51.3% for ZEA. The 39 barley samples were contaminated as follows: 35 samples (89.7%) with DON and NIV, 17 samples (43.6%) with DON, NIV, and 4-ANIV, 6 samples (15.4%) with DON, NIV, 4-ANIV, and 3,15-DADON, and 4 samples (10.3%) with DON, NIV, 4-ANIV, and 4,15-DANIV. Only one sample was contaminated with five 8-ketotrichothecenes (DON, NIV, 4-ANIV, 3,15-DADON, and 4,15-DANIV). ZEA was coincidentally found in 20 (51.3%) of 39 barley samples. In addition, the level of DON was positively correlated with that of NIV (r = 0.873) and the levels of NIV in the barley samples were always higher than those of DON.

As for the incidence of toxin contamination at a concentration of over 1,000 ng/g, 11 samples (28.2%) were contaminated with NIV and 1 sample was contaminated with trichothecenes. The mean concentrations of DON, NIV, and ZEA in positive samples were 170, 1,011, and 287 ng/g, respectively, and those of 3-ADON, 4-ANIV, and 4,15-DANIV were less than 100 ng/g. The maximal levels of toxins detected in barley were 1,051 ng/g for DON, 6,892 ng/g for NIV, 168 ng/g for 3-ADON, 71 ng/g for 4-ANIV, 28 ng/g for 4,15-DANIV, and 1,416 ng/g for ZEA.

**Table 1. Natural occurrence of trichothecenes and ZEA in barley from the southern part of Korea**

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>No. (%) of positive samples</th>
<th>Mean level (range) (ng/g) in positive samples</th>
</tr>
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<tbody>
<tr>
<td>DON</td>
<td>35 (89.7)</td>
<td>170 (25-1,051)</td>
</tr>
<tr>
<td>3-ADON</td>
<td>7 (17.9)</td>
<td>65 (13-168)</td>
</tr>
<tr>
<td>15-ADON</td>
<td>0 (0.0)</td>
<td>ND</td>
</tr>
<tr>
<td>3,15-DADON</td>
<td>0 (0.0)</td>
<td>ND</td>
</tr>
<tr>
<td>NIV</td>
<td>37 (94.9)</td>
<td>1,011 (39-6,892)</td>
</tr>
<tr>
<td>4-ANIV</td>
<td>17 (43.6)</td>
<td>25 (12-71)</td>
</tr>
<tr>
<td>4,15-DANIV</td>
<td>5 (12.8)</td>
<td>22 (15-28)</td>
</tr>
<tr>
<td>ZEA</td>
<td>20 (51.3)</td>
<td>287 (40-1,416)</td>
</tr>
</tbody>
</table>

* The trichothecenes were quantified by GC-MS with SIM, and ZEA was quantified by HPLC with a fluorescence detector.
* ND, not detected.
FIG. 1. Chromatograms of trimethylsilyl ethers of trichothecenes in barley and corn samples recorded by SIM. (A) Standard toxins: 3-ADON (a), 4-ANIV (b), and 15-ADON (c); (B) 3-ADON and 4-ANIV in barley samples; (C) 15-ADON and 4-ANIV in corn samples.

of contaminated samples were 65.2% for DON, 26.1% for 15-ADON, 34.8% for NIV, 10.9% for 4-ANIV, 15.2% for 4,15-DANIV, and 17.4% for ZEA. The 46 corn samples were contaminated as follows: 12 samples (26.1%) with DON and 15-ADON; 13 samples (28.3%) with DON and NIV; 6 samples (13.0%) with DON, NIV, and 15-ADON; 4 samples (8.7%) with DON, NIV, 4-ANIV, and 15-ADON; and 2 samples (4.3%) with DON, 15-ADON, NIV, and 4,15-DANIV. ZEA was coincidently found in 8 (17.4%) of 46 corn samples. The level of DON was positively correlated with that of 15-ADON (r = 0.961). The mean concentrations of DON and 15-ADON in positive samples were 310 and 297 ng/g, respectively, and the mean level of ZEA was 151 ng/g. Other trichothecenes were detected at concentrations of less than 100 ng/g. The maximal levels of toxins detected in corn were 2,752 ng/g for DON, 1,726 ng/g for 15-ADON, 366 ng/g for NIV, 139 ng/g for 4-ANIV, and 51 ng/g for 4,15-DANIV, and 388 ng/g for ZEA.

**Confirmation of 4,15-DANIV.** Although SIM analysis of some sample extracts indicated the presence of 4,15-DANIV, it was difficult to obtain the total ion chromatogram and a full mass spectrum of the toxin because of the relatively low concentration of 4,15-DANIV in the sample extracts. In order to unequivocally verify the presence of 4,15-DANIV, one corn sample which was positive for 4,15-DANIV by SIM analysis was chosen and prepared by the procedure described in Materials and Methods. The extract was then subjected to capillary GC-MS. Figure 2A shows the spectrum of the trimethylsilyl ether of the 4,15-DANIV standard. The mass spectrum of 4,15-DANIV gave the diagnostic ions at m/zs of 525, 450, 408, and 251 and the molecular ion at an m/z of 540. The mass spectrum of the trimethylsilyl ether of 4,15-DANIV from the corn extract is shown in Fig. 2B. The agreement of retention time coupled with the presence of diagnostic ions verified the presence of 4,15-DANIV in the sample extract.

**TABLE 2. Natural occurrence of trichothecenes and ZEA in corn from Kangwon province**

<table>
<thead>
<tr>
<th>Mycotoxin*</th>
<th>No. (%) of positive samples</th>
<th>Mean level (range) (ng/g) in positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>DON</td>
<td>30 (65.2)</td>
<td>310 (29-2,752)</td>
</tr>
<tr>
<td>3-ADON</td>
<td>0 (0.0)</td>
<td>ND*</td>
</tr>
<tr>
<td>15-ADON</td>
<td>12 (26.1)</td>
<td>297 (22-1,726)</td>
</tr>
<tr>
<td>3,15-DADON</td>
<td>0 (0.0)</td>
<td>ND*</td>
</tr>
<tr>
<td>NIV</td>
<td>16 (34.8)</td>
<td>77 (6-366)</td>
</tr>
<tr>
<td>4-ANIV</td>
<td>5 (10.9)</td>
<td>55 (23-139)</td>
</tr>
<tr>
<td>4,15-DANIV</td>
<td>7 (15.2)</td>
<td>29 (17-51)</td>
</tr>
<tr>
<td>ZEA</td>
<td>8 (17.4)</td>
<td>151 (4-388)</td>
</tr>
</tbody>
</table>

* The trichothecenes were quantified by GC-MS with SIM, and ZEA was quantified by HPLC with a fluorescence detector.

* ND, not detected.
DISCUSSION

The survey on barley samples randomly collected from farmers' stocks in the southern part of Korea revealed a heavy contamination of DON, 3-ADON, NIV, 4-ANIV, 4,15-DANIV, and ZEA. Among the five 8-ketotrichothecenes, the major toxins were NIV and DON rather than 3-ADON, 4-ANIV, and 4,15-DANIV. The incidences and mean levels of NIV and DON were much higher than those of the other trichothecenes. NIV and DON have been regular contaminants in Korean barley. However, the level of NIV detected in this study was much higher than that detected in previous reports (9, 10, 17, 18). The level of NIV was 5.9 times higher than that of DON. Recently, Park et al. (19) reported the natural occurrence of NIV and DON in the 1990 barley crop in Korea. The levels of two trichothecenes and ZEA detected in this study were similar to those detected in naked barley samples in their study. The levels of NIV in wheat and barley were also several times higher than those of DON in Japan (30) whereas DON was the major toxin in Canada, China, Poland, and Argentina (23). Thus, there are some differences in the level of the two trichothecenes in cereals in different countries.

The acute lethal toxicity of NIV is higher than that of DON; the 50% lethal doses of NIV in mice were 38.9 mg/kg of body weight (oral), 7.4 mg/kg (intraperitoneal), and 7.2 mg/kg (subcutaneous) (20), and those of DON were 46 mg/kg (oral), 70 mg/kg (intraperitoneal), and 45 mg/kg (subcutaneous) (25, 29). Other toxicological data, such as dermal toxicity, cytotoxicity, and inhibition of protein synthesis, showed that the toxicity of NIV is about 10 times higher than that of DON (27). The toxicity of barley with a NIV concentration of 1,011 ng/g would therefore correspond approximately to the toxicity of barley with a DON concentration of 10.11 mg/kg. This calculation simply indicates that the toxin levels in the 1990 crop are higher than the tolerance level of DON in uncleaned soft wheat at a level of 2,000 mg/kg in Canada.

The analytical results for Fusarium toxins in Table 2 indicate that corn samples were contaminated with DON, 15-NIV, NIV, 4-ANIV, 4,15-DANIV, and ZEA. The presence of NIV was also demonstrated in corn in Canada as a minor contaminant (24). Although the incidence of NIV was slightly higher than that of 15-ADON in Korean corn, the levels of DON and 15-ADON in corn samples seem to be more important than those of the other trichothecenes including NIV. Luo et al. (15) reported that three 8-ketotrichothecenes (DON, 15-ADON, and NIV) were contaminated in corn from high- and low-risk areas for human esophageal cancer in China. In their survey, the levels of DON and 15-ADON were also much higher than that of NIV. The mean level of 15-ADON in Korean corn was similar to that in Chinese corn from a high-risk area for esophageal cancer, though only 1 of 46 corn samples was contaminated with total DON (DON plus 15-ADON) at a level above 1,000 ng/g. The natural occurrence of 15-ADON with DON and ZEA was also reported in corn associated with feed refusal in the United States (1).

The acute lethal toxicity of 15-ADON is slightly higher than that of DON; the 50% lethal dose of 15-ADON in male mice was 34 mg/kg by oral routes (31). Much attention should be paid to the natural occurrence of 15-ADON as well as DON in Korean corn.

The present survey demonstrates that the pattern of the natural occurrence of monocetyl-DON and the ratios of NIV to DON in the two cereals were different for each cereal. There has been a remarkable difference in trichothecene production of Fusarium isolates from barley compared with those from corn in Korea (7, 12, 14, 21); most barley isolates of F. graminearum were NIV producers, but some of them were DON producers together with 3-ADON. None of the isolates tested were found to produce 15-ADON. On the other hand, the incidence of DON producers among the corn isolates of F. graminearum was higher than that of NIV producers. DON producers frequently coproduced 15-ADON, and 3-ADON producers were rare (7, 12). These chemotypes of trichothecenes in Fusarium isolates from barley and corn support our findings of the natural occurrence of trichothecenes in the two cereals. At this moment, we may conclude that there are regional differences in trichothecene production of Fusarium isolates between the barley-producing area and the corn-producing area even in the same country. A regional difference in the natural occurrence of trichothecenes was also observed in Japan (6). However, a host-related difference in the natural occurrence of trichothecenes by F. graminearum should not be ruled out; the barley-producing area is located only 300 km south of the corn-producing area in Korea. Thus, further surveys of the natural occurrence of trichothecenes in barley and corn from the respective areas are necessary in order to test the hypothesis of a host-related difference in trichothecene production.

It should be also stressed that the two cereals were contaminated with 4-ANIV and 4,15-DANIV, though the incidence and levels of the two toxins were low. The acute toxicity of 4,15-DANIV is 1.21 mg/kg (intraperitoneal) to 9.6 mg/kg (intraperitoneal) in mice (2, 5, 26). This is the first report of the natural occurrence of 4,15-DANIV in cereals. Additional surveys on the natural occurrence and further toxicological examination on 4,15-DANIV in cereals are expected to provide valuable informations on risk assessment of 4,15-DANIV.

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