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Production of 8-ketotrichothecenes by *Fusarium graminearum* on Corn and Barley

Yong Su Seo, Jeong Ah Seo, Hwang Bae Sohn and Yin Won Lee*
Division of Applied Biology and Chemistry and Research Center for New-Biomaterials in Agriculture, College of Agriculture and Life Sciences, Seoul National University, Suwon 441-744, Korea

옥수수와 보리에서 *Fusarium graminearum*의 8-ketotrichothecenes 생성

서영수 · 서정아 · 손황배 · 이인원*
서울대학교 응용생물화학부 및 농업생물신소재연구센터

ABSTRACT: The production of 8-ketotrichothecenes, deoxynivalenol (DON), nivalenol (NIV), and their monoacetyl derivatives was studied in rice and corn cultures using 8 isolates of *Fusarium graminearum* which were obtained from corn and barley samples. Higher concentrations of trichothecenes were produced on rice than corn, and production of the toxins on rice was enhanced by growing the fungi at 25℃. The isolates were used for evaluation of toxin production and pathogenicity after artificial inoculation to 5 corn and 3 barley cultivars. The kinds and the relative amounts of trichothecenes produced in cultures were consistent with those in infected kernels of corn and barley with some exceptions. As for DON chemotypes, the ratios of 15-acetyl-DON to 3-acetyl-DON were varied among the pathogen-cultivar interactions. The corn and barley cultivars showed the significant differences of resistance to the *Fusarium* isolates in disease severity and seedling blight, and resistance ranking to the different isolates was varied. However, significant correlations were observed between the total concentrations of trichothecenes in infected kernels of corn and barley and pathogenicities of the *Fusarium* isolates to their hosts.

Key words: **barley, corn, 8-ketotrichothecenes, *Fusarium graminearum*, pathogenicity.**

Fusarium graminearum Schwabe, the imperfect stage of *Gibberella zeae* (Schw.) Petch, is a fungal pathogen of cereals such as corn, wheat, and barley. It causes root rots and seedling diseases (5, 14), a head blight of wheat and barley, and stalk and ear rots of corn. This fungus produces many secondary metabolites including the mycotoxins, deoxynivalenol (DON), 15-acetyldeoxynivalenol (15-ADON), 3-acetyldeoxynivalenol (3-ADON), nivalenol (NIV), 4-acetylnivalenol (4-ANIV, fusarenon-X), as well as an estrogenic mycotoxin, zearalenone (ZEA). These mycotoxins have been found in cereals and animal feeds (1, 11-13, 25-27, 29) and are responsible for mycotoxicoses in farm animals and experimental animals (16). Especially the mycotoxins often encountered in cereals are DON, NIV, and ZEA in oriental countries (11-13, 25, 27, 29).

Corn is mainly produced in the Kangwon province which is located in the mideastern part of Korea and barley is produced in southern provinces, Chonbuk, Chonnam, Kyungbuk, and Kyungnam. The natural occurrence of 8-ketotrichothecenes in the two cereals was different (11). The major toxins in corn were DON and 15-ADON, and NIV and the contamination levels of DON and 15-ADON were higher than those of NIV. On the other hand, the major toxins in barley were NIV and DON and the contamination levels of NIV were usually higher than those of DON.

There was a remarkable difference in 8-ketotrichothecene production of *F. graminearum* isolates from corn compared to those from barley (20); the prominent chemotypes of corn- and barley-isolates were the DON-type and NIV-type, respectively. There appears to be an uneven distribution of the two chemotypes between the Kangwon province and the southern provinces of Korea. Such a regional difference of the two chemotypes was reported in Japan (8).

The primary objective of this study was to analyze several cultivars of corn and barley for the presence of 8-

*Corresponding author.

ketotrichothecenes after inoculation with *F. graminearum* isolates which were obtained from corn and barley samples. We wanted to know whether there was any difference in toxin production when corn was compared with barley and whether there was any correlation between the toxin production and resistance to corn ear rot or barley scab when corn and barley cultivars were inoculated with the two different chemotypes of *F. graminearum* isolates, the DON- and the NIV-chemotypes.

MATERIALS AND METHODS

Fungal isolates. A total of 8 isolates of *F. graminearum* were selected from the previous study (20) and used in this experiment. These isolates were deposited to Korean Collection for Type Cultures (KCTC) at Genetic Resources Center, Korea Research Institute of Bioscience and Biotechnology, Taejon, Korea and access numbers were given by KCTC. The isolates were stored in sterilized soil and recovered on potato dextrose agar as needed.

***In vitro* production of 8-ketotrichothecenes.** Erlenmeyer flasks (500 ml), each containing 100 g of rice or corn and 60 ml of distilled water, were autoclaved twice for 1 hr at 121℃ with a 24-hour interval. The rice or corn was inoculated with mycelium plugs from a 5-day-old potato dextrose agar of each isolate. In rice cultures, the flasks were incubated on two temperature regimes to examine the variation of trichothecene pro- duction; the flasks were incubated for 4 weeks at 25℃ and in the other treatment, the flasks were incubated for 2 weeks at 25℃ followed by 2 weeks for 5℃ (23). Corn cultures were grown for 4 weeks at 25℃. 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℃ until analysis.

Plant materials. The corn and barley cultivars used in this experiment are listed in Table 2 and 3, respectively. No information was available on the susceptibility of the corn and barley cultivars to inoculation with the pathogens. Five corn cultivars including Chalok (CO), Danok (DO), Hoengsung (HS), Kwangan (KA), and Suwon (SW) and three barley cultivars such as Oal (OL), Sachun (SC), and Tapgol (TG) were planted in field plots in the Experimental Farm, Seoul National University, Suwon, Korea. The corn cultivars were planted during the third week of April, 1996. The plots were consisted of four rows, 18 plants per row, replicated three times, and each plot was 0.2 m apart. Each row was 5.0-m long (80 cm between rows), the center 10 of which were inoculated. All of corn cultivars were harvested during the fourth week of August, 1996.

Of the three barley cultivars, winter barleys, OL and SC, were planted in field plots during the second week of October 1995, and spring barley, TG, was planted during the first week of March, 1996. Each plot was 3.0 m long, 0.3 m apart, and was replicated three times. Plots were fertilized and limed according to soil test recommendation.

Inoculation. Corn cultivars were inoculated as the silks turned brown, between 75 and 84 days after planting. Corn ears were inoculated in each replicate by inserting toothpicks colonized by the different isolates of *F. graminearum* into the center of the ear through the husks (7). Controls were inoculated with noninfested toothpicks. Toothpicks used for inoculation were boiled four times in distilled water to remove any toxic substances, autoclaved in potato dextrose broth, and stored for 2~3 days. Thirty to 50 toothpicks were placed in each petri dish (100 mm [inside diameter] by 15 mm) on potato dextrose agar and inoculated with a 4-mm mycelium plug from a 5-day-old culture of each *F. graminearum* isolate. Toothpicks were crisscrossed to provide adequate aeration, and cultures were grown under a 12-hour cycle of dark and light (5,000 lux) at 25℃ until heavy mycelial growth was evident.

For the inoculation to barley, conidia were prepared by inoculating 50 ml of sterile liquid carboxymethyl cellulose (CMC) in a 250 ml-Erlenmeyer flask with a small amount of soil from a soil culture of each *F. graminearum* isolate (4). The flasks were incubated for 7 days at 25℃ on a rotary shaker at 200 rpm. Macroconidia were harvested by pouring the CMC cultures through a 30 μm-mesh nylon screen to separate the conidia from the hyphal debris. The pellet was then centrifuged at 5,000 rpm for 10 min in sterile tubes. The pellet was resuspended in sterile water to a concentration of 10^6 conidia/ml. Twenty kernels in each replicate plot of the three cultivars were individually bagged with a paper, inoculated by spraying 2 ml of conidial suspension, and covered with plastic bags to preserve suitable moisture for 3~4 days after inoculation. All grain was harvested when the kernels were ripe (40 days after inoculation).

Each treatment in both corn and barley experiments was replicated three times, but analysis of 8-ketotrichothecenes within each treatment was made from a single subsample of the pooled replications.

Pathogenicity. Disease severity in corn was evaluated by using a six-class rating scale in which 1=less than 5%, 2=6 to 20%, 3=21 to 40%, 4=41 to 60%, 5=61 to 80%, 6=81 to 100% of the kernel exhibiting visible symptoms of infection such as rot and mycelium growth.

Seedling blight test was performed by sowing surface disinfected and germinated kernels of barley into petri dish filled with vermiculite (17). Mycelium grown for 7 days on potato dextrose agar in a petri dish was placed directly under the kernels, 2 cm below vermiculite. Ten days after sowing, the height of seedling was measured as compared to that of the control.

Mycotoxin analysis. Trichothecene mycotoxins, including DON, 15-ADON, 3-ADON, NIV, and 4-ANIV were purchased from Wako Chemical Co., Tokyo, Japan. Fugal cultures, corn, and barley samples were extracted by using a previously published procedure (24). Each ground sample 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 chloroform-methanol (9:1, vol/vol). The eluate was concentrated to dryness and the residue was redissolved in 2 ml of methanol.

A portion of each extract was reacted with trimethylsilylating reagent and analyzed with a JEOL JMS-AX 505 gas chromatograph-mass spectrometer (GC-MS) with a selected ion monitoring (SIM) mode. The analytical conditions were as follows: column, DB-5 fused silica column (0.25 mm [inside diameter] by 30 m, 0.25 μm film thickness, J & W Scientific, Folsom, CA, USA); column temperature, 120℃ for 5 min and then increased to 270℃ at 5℃/min; injector temperature, 280℃; ion source temperature, 200℃; interface temperature, 250℃; ionizing voltage, 70 eV; ionizing current, 300 μA; scanning rate, 2 sec/scan.

The *m/z* fragment ions monitored for quantification of 8-ketotrichothecenes were 422 and 393 for DON, 392 and 350 for 15-ADON, 377 and 287 for 3-ADON, 379 and 289 for NIV, and 480 and 450 for 4-ANIV. The calculation of trichothecene concentration was based on the average counts of the fragment ions of each standard toxin. The retention time for each toxin was 25.95 min for DON, 27.94 min for 3-ADON, 28.08 min for 4-ANIV, 28.47 min for 15-ADON, and 30.60 min for NIV. The detection limit of the method employed for the trichothecenes was ca. 5 ng/g.

RESULTS

***In vitro* production of 8-ketotrichothecenes.** Eight isolates of *F. graminearum* used in this experiment pro-

Table 1. Production of trichothecenes by the 8 isolates of *Fusarium graminearum* grown on rice and corn

Origin	Isolate	Mycotoxin	Concentration (μg/g)		
			Rice (A)[a]	Rice (B)[b]	Corn
Corn	KCTC 16656	Don	1,329	872	529
		15-ADON	102	56	49
		3-ADON	9	12	139
		NIV	7	5	10
		4-ANIV	ND	ND	ND
	KCTC 16657	DON	556	47	450
		15-ADON	1	8	44
		3-ADON	ND	36	ND
		NIV	ND	ND	ND
		4-ANIV	ND	ND	ND
	KCTC 16658	DON	971	83	104
		15-ADON	70	8	4
		3-ADON	8	9	3
		NIV	1	ND	ND
		4-ANIV	ND	ND	ND
	KCTC 16659	DON	1	111	362
		15-ADON	ND	90	25
		3-ADON	55	16	ND
		NIV	ND	ND	ND
		4-ANIV	ND	ND	ND
Barley	KCTC 16660	DON	1	1	27
		15-ADON	ND	ND	ND
		3-ADON	ND	ND	ND
		NIV	488	46	143
		4-ANIV	83	39	ND
	KCTC 16661	DON	1,960	1,702	117
		15-ADON	17	224	ND
		3-ADON	3,923	ND	8
		NIV	35	5	3
		4-ANIV	ND	ND	ND
	KCTC 16662	DON	2	9	1
		15-ADON	ND	ND	ND
		3-ADON	ND	ND	ND
		NIV	274	74	13
		4-ANIV	129	79	ND
	KCTC 16663	DON	3	5	6
		15-ADON	ND	ND	ND
		3-ADON	ND	ND	ND
		NIV	546	78	137
		4-ANIV	159	189	1

[a]Culture were grown for 4 weeks at 25℃.
[b]Culture were grown for 2 weeks at 25℃ followed by 2 weeks at 5℃.

duced different kinds and amounts of 8-ketotrichothecenes when grown on rice and corn (Table 1). The DON chemotypes (5 isolates) produced DON and monoacetyl-DON (15-ADON, 3-ADON, or both) when they were grown on rice or corn and could also produce low levels of NIV and 3, 15-diacetyl-DON. The NIV chemotypes (3 isolates) produced NIV and 4-ANIV as the major toxins and could produce low levels of DON. However, the concentrations of trichothecenes were affected by substrate and environmental temperature; higher concentrations of trichothecenes were produced on rice than corn, and production of the toxins on rice was relatively enhanced by growing the fungi at the room temperature rather than the low temperature.

Pathogenicity. The five corn cultivars showed the significant differences of resistance to corn ear rot in disease severity and significant differences among the *F. graminearum* isolates were also observed in disease severity (Table 2). The 8 isolates of *F. graminearum* were varied in pathogenicity; three (KCTC 16656, KCTC 16660, KCTC 16663) were highly pathogenic, two (KCTC 16657 and KCTC 16659) were moderately pathogenic, and three (KCTC 16658, KCTC 16661, and KCTC 16662) were weakly pathogenic. Among the five corn cultivars, KA was the most resistant and DO was the most susceptible to the *Fusarium* isolates.

Fusarium pathogenicity of barley was evaluated by a seedling blight test. A significant interaction was observed between the barley cultivars and the *Fusarium* isolates in seedling blight (Table 3). The *F. graminearum* isolates were grouped into three classes; three highly pathogenic isolates (KCTC 16656, KCTC 16661, and KCTC 16663), three moderately pathogenic (KCTC 16657, KCTC 16659, and KCTC 16660), and two weakly pathogenic isolates (KCTC 16658 and KCTC 16662). Among the three barley cultivars, TG was the most susceptible to the *Fusarium* isolates. Most isolates showed the similar pathogenicity in both corn and barley except for some isolates; the weakly pathogenic isolate to corn (KCTC 16661) was highly pathogenic to barley and the highly pathogenic isolate to corn (KCTC 16660) was moderately pathogenic to barley.

Table 2. Disease severity ratings of five corn cultivars inoculated with the 8 isolates of *Fusarium graminearum*

Corn cultivar[a]	Mean	*Fusarium* isolate	Mean
DO	4.00a	KCTC 16660	5.00a
SW	3.73ab	KCTC 16656	4.73a
HS	3.43b	KCTC 16663	4.53ab
KA	2.63c	KCTC 16659	3.60cd
CO	2.37c	KCTC 16657	3.33d
		KCTC 16661	2.27e
		KCTC 16662	1.93ef
		KCTC 16658	1.67fg
		Control	1.20g

Disease severity ratings were based on a scale of 1 to 6 where 1 = less than 5% of the earmoldy, 2 = 6~20%, 3 = 21~40%, 4 = 41~60%, 5 = 61~80%, and 6 = 81~100%.
Values followed by the same letter are not significantly different at the 5% level according to Duncan's test.
[a]DO; Danok, SW; Suwon, HS; Hoengsung, KA; Kwangan, and CO; Chalok.

Table 3. Seedling blight of three barley cultivars inoculated with the 8 isolates of *Fusarium graminearum*

Barley cultivar[a]	Mean	*Fusarium* isolate	Mean
OL	5.84a	KCTC 16661	1.97a
SC	5.59a	KCTC 16663	2.01a
TG	4.64b	KCTC 16656	2.59ab
		KCTC 16657	3.43b
		KCTC 16660	3.55b
		KCTC 16659	5.05c
		KCTC 16658	9.29e
		KCTC 16662	9.72e
		Control	9.95e

Seedling blight test was measured by the height (cm) of seedlings 10 days after germination.
Values followed by the same letter are not significantly different at the 5% level according to Duncan's test.
[a]OL; Oal, SC; Sachun and TG; Tabgol.

Trichothecene production on corn and barley after artificial inoculation. Kernels of corn and barley cultivars inoculated with the 8 of *F. graminearum* isolates were analyzed for 8-ketotrichothecenes. The analytical results of trichothecenes in corn and barley are shown in Tables 4 and 5, respectively. The production patterns of trichothecenes in infected corn cultivars were consistent in those in infected barley cultivars. The kinds of trichothecenes detected in infected kernels of the two hosts were same with those produced in the cultures. The total concentration of trichothecenes was very high in DO corn cultivar inoculated with the highly pathogenic isolates such as KCTC 16660 and KCTC 16663. On the other hand, the total concentration of trichothecenes was very high in TG barley cultivar inoculated with the highly pathogenic isolates such as KCTC 16656 and KCTC 16661. There were some variations of trichothecene production among pathogen-cultivar interactions; the highly pathogenic isolate (KCTC 16660) produced low levels of trichothecenes in SW corn cultivar whereas the weakly pathogenic isolate (KCTC 16661) produced the relatively large

Table 4. Trichothecene production in the 5 corn cultivars inoculated with *Fusarium graminearum*

Isolate	Mycotoxin	Concentration (μg/g) in the corn cultivars[a]				
		CO	DO	KA	HS	SW
KCTC	DON	761	291	313	142	524
16656	15-ADON	24	29	6	1	28
	3-ADON	14	39	1	3	76
	NIV	ND	3	ND	ND	6
	4-ANIV	ND	ND	ND	ND	ND
KCTC	DON	123	190	6	131	270
16657	15-ADON	3	1	1	3	1
	3-ADON	3	3	ND	ND	ND
	NIV	ND	1	ND	ND	ND
	4-ANIV	ND	ND	ND	ND	ND
KCTC	DON	22	54	ND	6	13
16658	15-ADON	1	2	ND	ND	ND
	3-ADON	ND	1	ND	ND	ND
	NIV	ND	ND	ND	ND	ND
	4-ANIV	ND	ND	ND	ND	ND
KCTC	DON	271	61	275	37	12
16659	15-ADON	5	1	37	1	ND
	3-ADON	3	2	ND	ND	ND
	NIV	ND	ND	1	ND	ND
	4-ANIV	ND	ND	ND	ND	ND
KCTC	DON	ND	8	1	1	ND
16660	15-ADON	ND	ND	ND	ND	ND
	3-ADON	ND	ND	ND	ND	ND
	NIV	9	2,846	344	128	3
	4-ANIV	1	1	2	2	3
KCTC	DON	ND	2	3	1	117
16661	15-ADON	ND	ND	ND	ND	ND
	3-ADON	ND	1	1	ND	2
	NIV	ND	ND	ND	ND	ND
	4-ANIV	ND	ND	ND	ND	ND
KCTC	DON	ND	10	ND	ND	ND
16662	15-ADON	ND	1	ND	ND	ND
	3-ADON	ND	ND	ND	ND	ND
	NIV	ND	329	ND	1	ND
	4-ANIV	ND	15	ND	ND	ND
KCTC	DON	1	2	ND	8	2
16663	15-ADON	ND	ND	ND	ND	ND
	3-ADON	ND	ND	ND	ND	ND
	NIV	606	1,266	1	94	68
	4-ANIV	10	6	ND	1	3
Control	DON	ND	1	ND	ND	ND
	15-ADON	ND	ND	ND	ND	ND
	3-ADON	ND	ND	ND	ND	ND
	NIV	ND	ND	ND	ND	ND
	4-ANIV	ND	ND	ND	ND	ND

[a]CO; Chalok, DO; Danok, KA; Kangwon, HS; Hoengsung, and SW; Suwon.

Table 5. Trichothecene production in the 3 barley cultivars inoculated with *Fusarium graminearum*

Isolate	Mycotoxin	Concentration (μg/g) in the barley cultivars[a]		
		OL	SC	TG
KCTC	DON	127	69	596
16656	15-ADON	8	2	15
	3-ADON	10	2	37
	NIV	ND	ND	ND
	4-ANIV	ND	ND	ND
KCTC	DON	209	87	486
16657	15-ADON	10	2	4
	3-ADON	7	3	7
	NIV	ND	ND	ND
	4-ANIV	ND	ND	ND
KCTC	DON	67	8	193
16658	15-ADON	2	ND	11
	3-ADON	3	ND	4
	NIV	0.1	ND	ND
	4-ANIV	ND	ND	ND
KCTC	DON	23	6	358
16659	15-ADON	1	ND	19
	3-ADON	1	ND	6
	NIV	ND	ND	ND
	4-ANIV	ND	ND	ND
KCTC	DON	0.1	ND	0.4
16660	15-ADON	ND	ND	ND
	3-ADON	ND	ND	ND
	NIV	21	64	80
	4-ANIV	ND	ND	ND
KCTC	DON	88	65	762
16661	15-ADON	ND	2	ND
	3-ADON	6	11	47
	NIV	ND	ND	ND
	4-ANIV	ND	ND	ND
KCTC	DON	ND	2	ND
16662	15-ADON	ND	ND	ND
	3-ADON	ND	ND	ND
	NIV	0.1	0.3	ND
	4-ANIV	ND	ND	ND
KCTC	DON	0.4	0.4	1
16663	15-ADON	ND	ND	ND
	3-ADON	ND	ND	ND
	NIV	36	40	193
	4-ANIV	ND	2	ND
Control	DON	ND	ND	ND
	15-ADON	ND	ND	ND
	3-ADON	ND	ND	ND
	NIV	ND	ND	ND
	4-ANIV	ND	ND	ND

[a]OL; Oal, SC; Sachun, and TG; Tabgol.

amounts of trichothecenes in the same cultivar. In barley, the weakly pathogenic isolate (KCTC 16658) produced more amounts of trichothecenes than the moderately pathogenic isolate (KCTC 16659) in OL cultivar.

DISCUSSION

In this experiment, we examined the production of 8-ketotrichothecenes both in cultures and in infected plants,

and confirmed the previous findings that *F. graminearum* isolates can be divided into DON and NIV chemotypes (9, 20, 23). We may conclude that there are regional differences in trichothecene production of *F. graminearum* isolates between the barley-producing area and the corn-producing area in Korea and host-related differences of trichothecene production should be ruled out.

As for the DON chemotype, some scientists (10, 18) suggested that 15-ADON and 3-ADON were produced independently by different DON-producing isolates of *F. graminearum* and that DON chemotypes could be subdivided into two types with respect to the production of monoacetyl-DON. However, the four DON-chemotypes could produce 15-ADON and 3-ADON in the cultures as well as in the field. Especially the ratios of 15-ADON to 3-ADON were varied among host cultivars in the field experiment. Sugiura *et al.* (23) reported that *Fusarium* isolates which produced both 15-ADON and 3-ADON could produce only 15-ADON at a low temperature condition. Hart *et al.*(7) also reported that 15-ADON was only identified in greenhouse-infected grain but not identified in field-inoculated grain. It seems that the final levels of trichothecenes and the ratios of 15-ADON to 3-ADON in infected grain might be determined by the complex interaction of fungal, host, and environmental factors. In this respect, differences in the production of monoacetyl-DON may not be chemotaxonomic characteristics of *F. graminearum*.

We studied pathogenicity and trichothecene production of *F. graminearum* to corn and barley. A significant correlation (r=0.737) was observed between the total concentration of trichothecenes in infected corn ears and disease severity and such a correlation (r=0.752) was also observed between the total concentration of trichothecenes in infected barley and the degree of seedling blight. Reid *et al.* (19) reported that visual evaluations of disease severity were highly correlated to DON concentration in the ear. In addition, correlations between pathogenicity and mycotoxin production of *Fusarium* species causing head blight were studied in wheat cultivars (3, 21, 28).

The role of trichothecenes in pathogenicity was studied by several authors (2, 6, 14, 15, 22, 28). Trichothecenes may increase the extent of disease symptoms and fungal colonization, but not involved in the primary interaction that determines the basic compatibility between host and pathogen.

Although the correlations between pathogenicity of *F. graminearum* isolates to corn and barley and the concentrations of trichothecenes in infected grains were demonstrated, there were some variations of trichothecene production among pathogen-cultivar interactions. It seems that disease resistance ranking to different isolates was varied and the differences of resistance could not be properly demonstrated by using a single isolate. Therefore, disease resistance could be defined properly only as an average reaction to different isolates and the pathogenicity of the isolates could be judged only as an average on several genotypes with different resistance.

요 약

옥수수와 보리로부터 분리한 8개의 *Fusarium graminearum* 균주들의 쌀과 옥수수 배양체에서 8-ketotrichothecenes인 deoxynivalenol(DON), nivalenol(NIV), 및 이들의 monoacetyl 유도체 생성을 조사하였다. Trichothecene의 생성은 옥수수배지보다 쌀배지에서 더 높았으며, 독소 생성의 온도는 25℃ 였다. 8개의 *F. graminearum* 균주들을 각각 5개의 옥수수 품종과 3개의 보리 품종에 인공 접종하여 식물체에서의 독소 생성과 병원성에 대하여 조사하였다. 대체로 배양체에서의 trichothecene 종류와 상대적 생성량은 접종한 옥수수와 보리에서도 같은 것으로 나타났다. DON 생성형 균주들의 15-acetyl-DON과 3-acetyl-DON의 생성률은 접종한 균주와 식물체 품종간에 따라 다양하게 나타났다. 옥수수와 보리 각 품종들은 8개의 *Fusarium* 균에 대하여 병 발생정도, 종자 감염율, 저항성 등에 있어서 다양한 변이를 나타내었으나, 감염된 옥수수와 보리에서의 trichothecene 총 농도와 기주에 대한 *Fusarium* 균주들의 병원성 사이에는 높은 상관관계를 보였다.

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