Production of mycotoxins by *Fusarium* isolates from scabby wheat harvested in Hokkaido, Japan

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杉浦 義紹^{*2}, 上野 芳夫^{*2}: 北海道産赤かび罹病小麦より分離した
Fusarium 属菌のマイコトキシン生産性

Summary

A total of 35 strains of *Fusarium* species were isolated from the scabby wheat grains harvested in Hokkaido which have been found to be contaminated with deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEN), and were examined on their producibility of mycotoxins such as trichothecenes and ZEN.

DON and/or ZEN-producers of *Fusarium graminearum* and NIV and/or ZEN-producers of *F. poae* were detected. This is the first finding that *F. poae* produces NIV in rice culture.

In the preceding paper¹⁾, we have revealed the co-contamination of *Fusarium* mycotoxins, viz. nivalenol (NIV), deoxynivalenol (DON) and zearalenone (ZEN), in scabby wheat grains harvested in 1984 at Tokachi district of Hokkaido, Japan. In this communication, we aimed to clarify thier mycological occurrence.

Materials and Methods

Three scabby wheat samples (sample Nos. H4, H8 and H10), which have been found to be contaminated with 2090-4900 ng/g of DON, 125-435 ng/g of NIV and 77-95 ng/g of ZEN, were used for mycological survey. After surface-sterilization

with 1 % sodium hypochlorite solution for 1 min, the grains were plated on potato-dextrose agar (PDA) or Czapek-Dox agar supplemented with 0.01% chloramphenicol in Petri dishes. The plates were incubated at 25°C for 7 days, and the developing fungi were isolated. The colonies of *Fusarium* species were singly transferred to PDA slants. The identifications of *Fusarium* isolates were carried out by the method of Booth²⁾, and the producibility of *Fusarium* mycotoxins was performed according to the previous report³⁾, as follows.

Each of *Fusarium* isolates was cultured at 25°C for 21 days in 50 g of polished rice grains supplemented with 16 ml of water. The artificially molded rice was dried in a hot-air drying oven at 45°C for 40 hr, and finely powdered in a blender. *Fusarium* mycotoxins in the molded rice were extracted and purified by the previous method⁴⁾ summarized briefly as follows. The samples (5 g) were extracted with acetonitrile-water (3:1, v/v), and the extracts were defatted with n-hexane. After an addition of ethanol to the aqueous

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acetonitrile layer, the solvents were evaporated on a rotary evaporator. The residue was purified by a chromatographic procedure using a Florisil column. *Fusarium* mycotoxins such as DON, NIV, ZEN, T-2 toxin (T-2), diacetoxyscirpenol (DAS) and neosolaniol (NS), in column eluates were derivatized to trimethylsilyl ethers, and their amounts were estimated by gas chromatography-mass spec-

trometry (Hitachi Model M-80A, Hitachi Ltd., Tokyo) with selected ion monitoring mode. The detection limits were 50 ng/g for DON, NIV and ZEN, and 100 ng/g for T-2, DAS and NS.

Results and Discussion

As shown in Table 1, a total of 35 strains of *Fusarium* species were isolated from the scabby

Table 1. Production of mycotoxins by *Fusarium* isolates from wheat harvested in Hokkaido

Species	Strain No.	Production of mycotoxins* ¹ (ng/g)		
		DON	NIV	ZEN
<i>F. equiseti</i>	H8F14	ND* ²	ND	ND
<i>F. graminearum</i>	H4G1	290	ND	270
"	H4G2	290	ND	5300
"	H4G20	140	ND	3200
"	H8G3	230	ND	5300
"	H8G4	2000	ND	8000
"	H8G5	15000	ND	1800
"	H8G6	50	ND	1200
"	H8G7	ND	ND	50
"	H8G8	430	ND	5800
"	H8G9	400	ND	14500
"	H8G10	ND	ND	610
"	H8G11	1300	ND	63900
"	H8G12	400	ND	26100
"	H8G13	200	ND	1700
"	H8G14	370	ND	16000
"	H8G15	140	ND	980
"	H8G16	4900	ND	6200
"	H8G17	1300	ND	28000
"	H10G18	60	ND	1300
"	H10G19	900	ND	81000
<i>F. poae</i>	H4F9	ND	380	310
"	H4F10	ND	130	ND
"	H4F11	ND	250	ND
"	H4F12	ND	80	ND
"	H4F13	ND	130	ND
"	H8F18	ND	340	ND
"	H8F20	ND	ND	ND
"	H8F21	ND	80	ND
<i>F. tricinctum</i>	H8F19	ND	ND	ND
<i>Fusarium</i> sp.	H8F15	ND	460	ND
"	H8F16	110	ND	ND
"	H8F17	230	ND	ND
"	H8F22	110	ND	ND
"	H8F23	ND	ND	ND

*¹ DON: deoxynivalenol, NIV: nivalenol, ZEN: zearalenone.

*² ND: not detected (the limits of detection are 50 ng/g for DON, NIV and ZEN).

wheat and they were identified as *F. graminearum* (20 strains), *F. equiseti* (1 strain), *F. poae* (8 strains), *F. tricinctum* (1 strain) and *Fusarium* sp. (5 strains).

Their producibility of *Fusarium* mycotoxins in rice culture was as follows. Among 20 strains of *F. graminearum*, 18 produced DON in range of 50–15,000 ng/g and 20 produced ZEN in range of 50–81,000 ng/g. The average productions of DON and ZEN by the toxin-producing strains were estimated to be 1,578 and 1,356 ng/g, respectively. The present data supported the finding of Ichinoe et al.⁵⁾ that *F. graminearum* in the wheat collected from Hokkaido produced DON.

Interestingly, in addition to the high producibility of *F. graminearum* for DON and ZEN, 7 out of 8 strains of *F. poae* produced 80–380 ng/g of NIV, among which one isolate also produced ZEN. This is the first finding that *F. poae* produces NIV in rice culture. The details of morphological features of the *F. poae* strains will be reported elsewhere.

No production of DAS, T-2 and NS was encountered on the strains in the present survey.

To summing up the above results, the scabby wheat grains from Hokkaido have been infected

with DON and ZEN-producers of *F. graminearum* and NIV and/or ZEN-producers of *F. poae*. Thus the DON and ZEN contaminations occurred in the scabby wheat mainly resulted in the invasion of *F. graminearum*, while the presence of NIV seems to be caused by *F. poae*.

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