PATHOGENICITY AND MYCOTOXIGENICITY OF FUSARIUM SPP. IN WHEAT

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During 1983 in Hokkaido northern Japan, winter wheat encountered unusually cool and wet weather conditions during the period of ripening, and was severely infected with the head blight pathogen, Fusarium graminearum.

The epidemic of head blight that occurred in the central Hokkaido in 1983 was unusually widespread and destructive. The wheat samples were collected at different areas of central Hokkaido for chemical and mycological analyses.

A total of 13 samples with no visible discoloration or damage come from farmers stocks during first week of harvested in August in 1983. In addition, 19 wheat samples were taken from one of the sampling sites where the cereals were heavy damaged by head blight. Prior sampling, these infected wheat had been left standing in the fields. The grains failed dry down effectively and commonly sprouted in the ear, and also sometimes purpurish-black perithecia appeared on the spikelets and stems.

Chemical analysis of the harvested wheat grain revealed the presence of trichothecene mycotoxin, deoxynivalenol (DON) in samples of crops from 1983. The concentrations of the naturally occurring DON varied from 0.18 to 5.96 µg/g, and the infection rate of F. graminearum ranged from 4 to 78% in these samples by mycological examination.

The chemical assay did not identify any other trichothecenes including nivalenol and T-2 toxin in the wheat samples.

After single spore isolation, we have tested isolates of F. graminearum obtained from DON contaminated wheat samples for production of mycotoxin. Analysis of the data on trichothecene production, showed that isolates of F. graminearum produced DON and its monoacetate.

The production of these mycotoxins in rice culture of the isolates was examined by TLC and ECD-GC according to analytical method of Kamimura et al. (Ref. 1).

Results from DON and mycological examination showed that concentraion of DON and infection levels were considerably lower in 13 samples than heavily damaged 19 samples. These results suggested that there are two types of DON-producing strains of F. graminearum, DON and 3-acetylDON and DON and 15-acetylDON producing strains(Table 1). It is interesting to notes that fungi producing the two different DON compounds are mixed in the same sample.

Table 1. Occurrence of deoxynivalenol and its producing F. graminearum in wheat grains in Hokkaido

Sample	DON level ($\mu\text{g/g}$)	<u>Fusarium graminearum</u>				
		% of infection	Strain no.	<u>Trichothecene produced</u>		
				DON	3AcDON	15AcDON
83-HW-2	0.77	12	F-1491	+		+
			F-1492	+		+
			F-1493	+	+	
83-HW-3	3.95	6	F-1494	+	+	
			F-1495	+	+	
			F-1496	+		+
83-HW-12	0.83	10	F-1507	+		+
			F-1508	+	+	
			F-1509	+	+	
83-HW-13	0.18	10	F-1510	+		+
			F-1511	+		+
			F-1512	+	+	

Table 2 shows the production of trichothecenes by Gibberella zeae isolated from perithecia formed on severely damaged wheat heads and ears. Using single ascospore isolation technique, the isolates could also be divided into two types: DON-3AcDON and DON-15AcDON type.

Table 2. Deoxynivalenol and its monoacetate production by G. zeae isolated from scabby wheat head

Sample	Strain no.	<u>Trichothecene produced</u>		
		DON	3AcDON	15AcDON
83-HDW-1	F-1559	+	+	
	F-1560	+	+	
	F-1561	+	+	
83-HDW-2	F-1564	+	+	
	F-1565	+	+	
	F-1566	+	+	
83-HDW-3	F-1569	+		+
	F-1570	+		+
	F-1571	+		+
83-HDW-14	F-1574	+	+	
	F-1575	+	+	
	F-1576	+	+	

In field trials, inoculation of the wheat by spraying with spore suspension of Fusarium spp. to heads at the flowering stage was performed (Ref. 2). In the present paper we have also attempted to examine the pathogenicity of Fusarium species from wheat samples harvested in Hokkaido and elsewhere.

They were examined using field inoculation test with wheat cultivar 'Omase komugi' which is susceptible to head blight. The symptoms appear in 10 - 15 days when kept at a highly humid condition after inoculation.

Selected isolates of Fusarium spp. were used to examine their pathogenicity to wheat. The strains were chosen on the basis of trichothecene production in culture. After two months, the heads were harvested and evaluating their pathogenicity, rated for percent infection on spikelets, weight of 1000 grains was determined, and the infected grains were stored at 4°C prior to chemical analysis.

For all of the examined species, the most pathogenic species was F. graminearum, followed F. avenaceum, F. acuminatum and F. sporotrichioides.

By inoculation test, F. graminearum and F. avenaceum caused head blight of wheat. Yield loss was severe in order by inoculation with same amount of inoculum. F. acuminatum and F. sporotrichioides showed the similar symptom, however yield loss was not detected by both species.

Table 3 shows the results of two representative strains of F. graminearum and F. acuminatum. In addition, the influence of fungicide (Thiophanatemetyl) on the results were determined as shown.

Table 3. Profile of infection and effects of fungicide on field-inoculated wheat

Tested fungi	Infected spikelets	Yield of grains*	Weight of 1000 grains	Wt. of 1000 poorly grains
Non-treatment:				
<u>F. graminearum</u> (F-744)	78.2 %	20.6 g	29.8 g	4.9 g
<u>F. acuminatum</u> (F-1150)	18.2	69.2	37.1	5.3
Control	4.2	69.8	36.9	-
Fungicide spraying:				
<u>F. graminearum</u> (F-744)	8.3	71.0	38.5	5.7
<u>F. acuminatum</u> (F-1150)	5.4	72.6	40.5	5.2
Control	0.5	66.1	39.6	6.5

* Total weight of yield grains per one stubble

Trichothecenes in the field-inoculated wheat grains were analyzed by procedures employed in a similar assay method used for naturally contaminated wheat grains. There is a disagreement between the analytical results of the field-inoculated grains and results of the survey (Table 4).

Table 4. Trichothecene formation by F. graminearum in grains of field-inoculated wheat

Strain no.	Trichothecene (µg/g)		
	DON	3AcDON	15AcDON
F-1160	25.4	0.4	—
F-1170	26.0	—	0.9
F-1395	18.0	0.4	—
F-1396	5.4	0.2	—
F-1410	13.0	0.3	—
F-1411	57.1	0.7	—

Although DON was detected only naturally infected grain, it is more likely that the co-existence of 3-AcDON or 15-AcDON, found under laboratory conditions, in field-inoculated grains occurred as minor products.

Isolates of F. acuminatum produced trichothecenes in liquid culture yielding T-2 toxin, HT-2 toxin, diacetoxyscirpenol and neosolaniol (Ref. 3), whereas in field-inoculated wheat grains, only T-2 toxin and HT-2 toxin were found. In the field-inoculated grain, HT-2 toxin was always found in higher amounts than T-2 toxin (Table 5).

Table 5. Trichothecene formation by F. acuminatum in grains of field-inoculated wheat

Sample no.	Trichothecenes in grain (µg/g)			
	T-2	HT-2	DAS	NS
F-1150-1.9*	2.29	7.45	ND	ND
F-1150-2.2	0.36	2.79	ND	ND
F-1150-2.5	0.14	1.83	ND	ND
F-1150-2.8	0.09	1.72	ND	ND

* type of kernel sieve size

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