BIOINTERCONVERSION OF TRICHOTHECENE MYCOTOXINS BY TOXIGENIC FUSARIUM SPECIES
—ITS POSSIBLE INVOLVEMENT IN NATURAL CO-OCCURRENCE OF DEOXYNIVALENOL AND NIVALENOL

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SUMMARY

On the basis of data obtained from an one-grain analysis, the distribution of deoxynivalenol (DON) and nivalenol (NIV) in wheat and barley is considered to be heterogeneous. *Fusarium graminearum* strains capable of producing either DON or NIV were found in cereal grains harvested from the same field, indicating that both types of *F. graminearum* simultaneously occur in crop fields and independently produce the individual toxins in cereals. NIV-producing *F. graminearum* was proved to be able to convert DON into NIV and its acetate not only in liquid media but also in solid media. From these results, it is likely that the microbial transformation of DON to NIV by NIV-producers may be an alternative route for the generation of NIV in cereal grains.

INTRODUCTION

DON and NIV are two major trichothecene mycotoxins occurring naturally in cereals, especially in small grains. These mycotoxins were first found in wheat and barley grains in our laboratory (ref. 1). From our field surveys carried out thereafter, we concluded that both toxins coincidentally occur in wheat and barley of the southern Japan at considerably high incidence, regardless of crop year, habitat, hybrids and variety of the cereals (ref. 2). More recently, several papers have reported the natural co-occurrence of DON and NIV in other countries (refs. 3-4).

However, very few papers have described on the mechanism of the co-occurrence of both toxins. Natural contamination of DON and NIV is generally expected to be resulted from plant-fungi and fungus-fungus interactions on host plants, and mode of the co-occurrence will be classified into three categories as follows: (1) independent generation of DON and NIV on host plants by individual toxin-producers, (2) microbial conversion of DON into NIV by fungi including NIV-producers, and (3) modification of DON by enzymes of host plants to elaborate NIV.

In this paper, the author discuss on the mode of natural co-occurrence of DON and NIV in small grains, and postulate that fungus-fungus interaction on host plants possibly results in microbial conversion of DON into NIV on plants.

INDEPENDENT GENERATION OF DON AND NIV

Of 106 isolates in total, predominantly *F. graminearum* which were isolated from wheat and barley samples of different origins of the southern Japan, 43 isolates produced either DON or NIV (ref. 5). Furthermore, as shown in Table 1, 41 isolates out of 85 isolates from three different fields were also found to be capable of producing either DON or NIV in laboratory cultures. In addition, it is worth noting that either DON- or NIV-producers of *F. graminearum* were isolated from the same field. These facts indicate that different strains of *F. graminearum* capable of producing either DON or NIV simultaneously invade field crops before harvest.

Table 1. Incidence of trichothecene-producing *F. graminearum* in crop fields of wheat and barley in Okayama, Japan (July, 1983)

Crop field	No. of isolates tested	No. of isolates producing		No. of non-producing isolates
		DON	NIV	
IABS-South	47	3	14	30
IABS-North	12	4	5	3
SOJA	26	3	12	11
Total	85	10	31	44

On the other hand, approximately equal levels of DON and NIV are usually detected in the cereals of the southern Japan when grain samples are analyzed as a mass or as a lot level (refs. 2 & 6). In order to clarify whether both DON and NIV are present in individual grains, a simple and rapid method was devised for one-grain analysis. This method (named 'DOG' analysis) consists of a double-layered chromatography of alumina and Florisil (ref. 7).

Of 102 grains in total collected by random sampling, 50% of the grains were contaminated with the toxins. Contamination % was below 40% for mature grains, as high as over 70% for other grains, and 100% for the coloured grains (Table 2). With respect to the contamination profile of DON and NIV, the wheat grains were classified into three groups : grains contaminated with DON, those with NIV, and those with both toxins at a ratio of 9 : 22 : 20 in numbers, respectively. Based on these figures, it is suggested that the former two groups were invaded with either DON- or NIV-producer whereas the latter was with both producers, thereby resulting in the heterogenous occurrence of DON and NIV in this grain sample.

On the other hand, the heterogenous distribution of the toxins implies that the third category, i.e. the conversion of DON into NIV by plant enzymes should be ruled out.

Table 2. One-grain analysis of a wheat sample contaminated with DON and NIV

Appearance of grains	No. of grains	No. of contaminated grains (%)	No. of grains contaminated with		
			DON	NIV	DON & NIV
Immature	10	8 (80)	2	3	3
Shrunk	4	3 (75)	0	1	2
Insect-damaged	4	3 (75)	0	1	2
Reddish	9	9 (100)	0	1	8
Mature	75	28 (37)	7	16	5
Total	102	51 (50)	9	22	20

MICROBIAL CONVERSION OF DON INTO NIV

Considering the fact that some grains, but not all, were coincidentally infested with both producers, the question is raised whether DON is converted into NIV by NIV-producers in same grains. In order to confirm this possibility (i.e. the second category), microbial conversion of DON and related derivatives was attempted.

F. graminearum No. 1383, a strain producing NIV and its acetate (F-X, 4,15-AcNIV), was incubated with reciprocal shaking in yeast extract-polypeptone-sucrose media (pH 7.0) for 4 days at 28 C. Washed mycelia (2 g wet wt.) was resuspended in 50 ml of phosphate buffer (67 mM, pH 6.8) containing [14 C]DON followed by incubation for 24 hr. The reaction products were analyzed by reversed-phase HPLC radiochromatography. As shown in Table 3, as high as 82% of the added substrate was efficiently converted into NIV and its acetates within 24 hr. In addition, these products were also obtained by incubating DON acetates such as 3-AcDON, 15-AcDON and 3,15-AcDON under the above condition.

For the purpose of elucidating the possible occurrence of this reaction on cereal grains, the transformation was carried out on a solid phase using polished rice spiked [14 C]DON. After incubation of *F. graminearum* No.1383 for 24 days at 27 C, the substrate was completely metabolized to afford NIV (24% of the products), F-X (72%) and 4,15-AcNIV (4%).

From these evidences, it seems highly possible that NIV occurring naturally in cereal grains may be generated by an alternative route, i.e. by the microbial conversion of DON into NIV by NIV-producers. Furthermore, as reported by Baldwin *et al.* (ref. 8), diacetoxyscirpenol producer *Fusarium* sp. used in this study was also able to transform DON to NIV and its acetates. This fact postulates that some of fungi other than NIV-producing *Fusaria* may also contribute to the elaboration of NIV from DON if they encounter with DON-producing fungi and/or DON itself on cereal grains.

This microbial hydroxylation at nonactivated 4 β -position of DON is particularly interesting event from the view point of both biogenesis of NIV and evolutionary

relationship between DON-producer and NIV-producer.

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Table 3. Bioconversion of [14 C]DON by *F. graminearum* No.1383

Metabolite	%, Transformation		
	Experiment 1	Experiment 2	Average
DON	13.4	13.4	
3-AcDON	4.0	4.4	17.6
NIV	11.1	13.2	
4-AcNIV (F-X)	67.6	65.7	
4,15-AcNIV	3.9	3.4	82.4

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