ニバレノール及びデオキシニバレノール産生性Fusarium spp.の選抜への酵素免疫測定法の応用

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Application of an enzyme-linked immunosorbent assay for the screening of *Fusarium* spp. producing nivalenol and deoxynivalenol

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Summary

Two monoclonal antibodies KTM-205-POD and KTM-240-POD which are highly specific to 3,4,15-triacetyl-NIV (TANIV) and TANIV + 3,15-diacetyl-DON (DADON), respectively, were used for the determination of nivalenol (NIV) and deoxynivalenol (DON) production by *Fusarium* spp. Sixteen strains of *F. graminearum* isolated from wheat and barley grains were cultured on polished rice substrate. NIV, DON and their acetylated derivatives present in the culture extract were partially acetylated into TANIV and DADON, respectively, before direct competitive enzyme-linked immunosorbent assay (ELISA). Results determined by ELISA, expressed as NIV and DON equivalents, were subsequently confirmed by gas chromatography-mass spectrometry. The correlation between the two methods for concentrations of total NIV and total DON was r^2 = 0.9875 ($p<0.001$). The results show that ELISA, at a cut-off limit of 25 mg/kg, can be used as a rapid and simple method for the screening of NIV- and DON-producing strains of *F. graminearum*.

Key words : ELISA, nivalenol, deoxynivalenol, *Fusarium graminearum*

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Introduction

Fusarium graminearum (sexual state: *Gibberella zeae*) causes crown rot and head blight of wheat and barley and stalk rot of maize[1]. Head blight can be a devastating disease affecting all classes of wheat and other small grains throughout the world[2]. In Japan, *F. graminearum* predominates among several species causing *Fusarium* head blight disease[3]. This disease reduces yield and quality of grains, and the harvested grain is often contaminated by mycotoxins, constituting a potential health risk for animals and humans. Several outbreaks of *Fusarium* invasion and toxicoses in humans and farm animals have occurred in Japan[4].

Nivalenol (NIV) and deoxynivalenol (DON) are the most important trichothecene mycotoxins produced by *F. graminearum*. The co-occurrence of NIV and DON in wheat and barley grains is common and widespread in Japan[5]. Based on its trichothecene profile, *F. graminearum* was divided into two distinct groups: the DON-type strains produce DON and acetyl-DON, and the NIV-type strains produce NIV and 4-ANIV[6]. DON producers are more dominant in China, North Europe and South and North America[7,8], whereas NIV producers have been reported in several countries of Africa, Asia and Europe and are the dominant strains found in Korea. In Japan, occurrence of the two

types appears to differ geographically[5]. NIV producers are predominant in Honshu with the exception of the northern Tohoku and western Chugoku districts. In contrast, DON producers are predominant in northern Tohoku, Hokkaido and Kyushu districts[9]. Furthermore, DON producers were also classified into two subtypes: 3-ADON and 15-ADON producers. In Japan mainly 3-ADON strains are found but 15-ADON strains are often isolated in the northern districts[5].

DON exerts phytotoxic effects that are involved as a virulence factor in the pathogenicity of *F. graminearum*[10] whereas, NIV exhibits greater mammalian toxicity[11], thus the prevalence of each producer is important in determining the toxicity of infected kernels, especially in areas where both producers occur simultaneously.

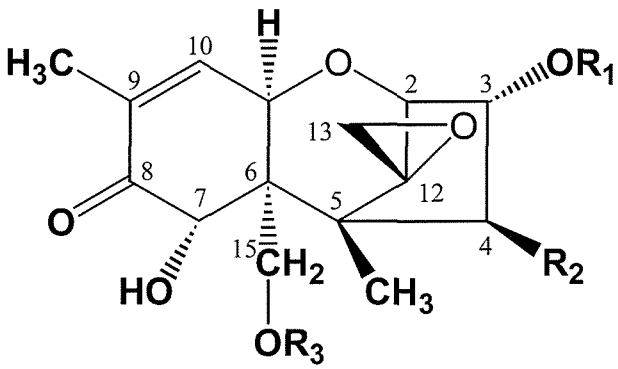
Physicochemical methods such as gas chromatography-mass spectroscopy (GC-MS), and high-pressure liquid chromatography (HPLC) are currently used for screening DON- and NIV-producing *F. graminearum* but are time-consuming, expensive and require extensive and complicated sample cleanup prior to analysis. Enzyme-linked immunosorbent assays (ELISA) offer the advantages of being fast, specific, sensitive and inexpensive, and could be used for screening a large number of samples. However, at present only an ELISA assay to screen DON is commercially available. To overcome this limitation, our laboratory developed two monoclonal antibodies: KTM-205-POD against 3,4,15-triacetyl-NIV (TANIV) and KTM-240-POD against TANIV + 3,15-diacetyl-DON (DADON) to determine total NIV and/or total DON after samples were partially acetylated. The objective of this study was to screen NIV- and DON-producing *F. graminearum* using a direct competitive ELISA and to examine the accuracy and precision of this method for use as a routine screening test.

Materials and Methods

Reagents and mycotoxin standards All organic and inorganic chemicals were of analytical grade quality. Unless otherwise noted, all reagents were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Trichothecene standards listed in Fig. 1 were prepared in our laboratory (>97 % purity determined by GC-MS and HPLC). For ELISA, phosphate-buffered saline (PBS) solution consisting of 10 mM sodium phosphate buffer (pH 7.4) with 140 mM NaCl was prepared, and ovalbumin (OVA) and thimerosal were obtained from Sigma Chemical Co. (St. Louis, MO, USA). For the derivatization of trichothecenes to trimethyl-silylated ethers (TMS), a solution of *N*-TMS-imidazole: *N,O*-bis-TMS-acetamide: TMS-chloride (3:3:2, v/v/v) was used.

Culture of Fusarium strains The sixteen strains of *F. graminearum* used in the present study were isolated from wheat and barley grains harvested at Kagawa University Farm, western Japan in 1999. All the strains were maintained as single-spore cultures using the procedure of Nelson *et al.*[1]. The polished rice (1 kg) used as a substrate was soaked in water for 2 h, drained thoroughly and then autoclaved. Thirty grams of the rice substrate were dispensed into a 300 ml flask, added with deionized water to achieve 40 % (w/w) moisture based on the original weight of the substrate and autoclaved again. A plug of mycelium (0.5 cm diameter) from 10 day-old PDA slant culture of each strain was introduced into each flask. The cultures were incubated at 25 ℃ in darkness, and harvested after two weeks and stored at -30 ℃ until toxin analysis.

Extraction Trichothecenes were extracted by homogenizing 5 g polished rice culture with 20 ml



Trichothecene	R_1	R_2	R_3
DON	H	H	H
3-ADON	Ac	H	H
15-ADON	H	H	Ac
DADON	Ac	H	Ac
NIV	H	OH	H
4-ANIV	H	OAc	H
4,15-DANIV	H	OAc	Ac
TANIV	Ac	OAc	Ac

Ac: $COCH_3$ (acetate).

Fig. 1. Chemical structure of nivalenol (NIV), deoxynivalenol (DON) and their acetylated derivatives used in the present study.

methanol:water (3:1, v/v), shaking for 1.5 h and centrifuging (1400 × g) for 15 min. An aliquot (80 μl, equivalent to 0.02 g) of the supernatant was air-dried and kept under vacuum for at least 12 h, then assayed using ELISA. For TLC and GC-MS analysis, 2 ml (equivalent to 0.5 g culture) supernatant was evaporated to dryness, redissolved in 0.5 ml methanol followed by addition of 4.5 ml chloroform. The solution was applied to a glass mini-column packed with florisil (0.5 g, 60-100 mesh) and topped with anhydrous Na_2SO_4 (0.3 g), and eluted twice with 4 ml chloroform:methanol (9:1, v/v). The eluate was evaporated to dryness, and the residue was redissolved in 25 μl and 0.5 ml methanol for TLC and GC-MS analysis, respectively.

ELISA ELISA analysis was conducted according to the method reported by Ikeda *et al.*[12] and Yumbe-Guevara and Yoshizawa[13]. NIV- and DON-related trichothecenes present in the sample residue were partially acetylated into TANIV and DADON by adding dried pyridine (50 μl) and acetic anhydride (25 μl) (Fig. 2). The samples were heated at 45 ℃ for 40 min, air-dried at room temperature, re-dissolved in 0.1 ml ethanol, and diluted with 0.9 ml PBS containing 0.06 % (v/v)

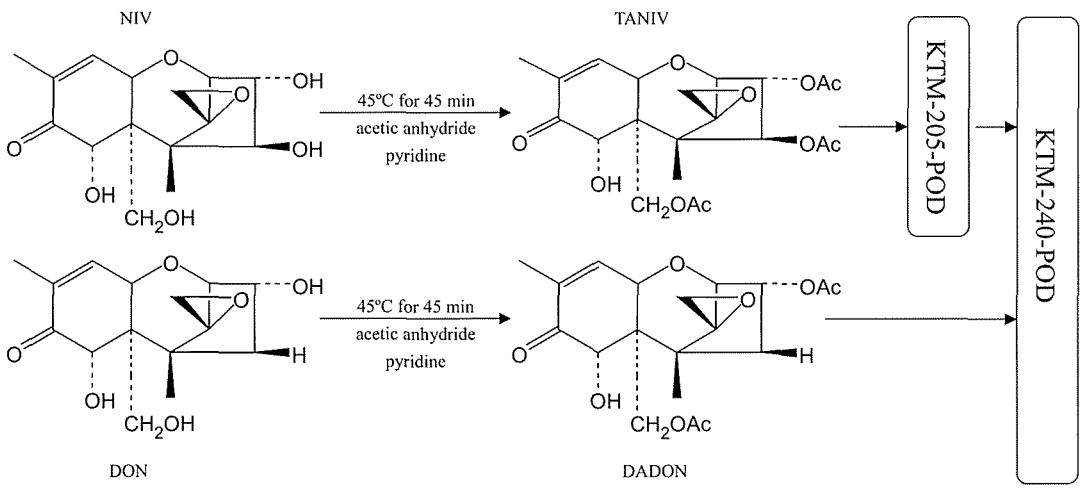


Fig. 2. Partial acetylation of NIV and DON into TANIV and DADON for ELISA analysis using two monoclonal antibodies (KTM-205-POD and KTM-240-POD).

Tween 20 (PBST).

The antigen (4,15-diacetyl-NIV-3-*O*-hemisuccinate-OVA) was dissolved in a 0.1 M $NaHCO_3$-Na_2CO_3 coating buffer (pH 9.6) to yield a concentration of 0.4 μg/ml, and 100 μl of this solution was applied to each well of a Costar 96 flat bottom microtiter plate (Corning Incorporated, NY, USA). Microtiter plates were shaken in a microplate mixer MPM-1 (Iwaki Glass Co., Tokyo, Japan) for 1 min and incubated at 4 ℃ overnight. Unbound conjugate was removed by washing the plates three times with PBST (Immuno washer NK-300, Inter Med Japan, Tokyo, Japan) and then carefully tapping them dry. Nonspecific binding was minimized by adding 200 μl/well blocking solution (0.1 % skim milk and 0.05 % thimerosal in PBS), washing 3 times with PBST and tapping the plates dry. Fifty microliter TANIV standard with concentrations ranging from 0.1 to 10 ng/ml in 10 % ethanol-PBST or 50 μl acetylated sample diluted in 10 % ethanol:PBST (1:10000) was added per well, and mixed with 50 μl peroxidase conjugate antibody solution. KTM-205-POD (100 ng/ml) or KTM-240-POD (150 ng/ml) antibodies diluted in 1 % OVA in PBS were used against TANIV or TANIV + DADON, respectively. The plates were shaken for 1 min, incubated at 4℃ overnight, washed six times, and tapped dry before using. Enzyme substrate solution was prepared by dissolving 100 μl 3,3',5,5'-tetramethyl benzidine (TMBZ) and 100 μl of 0.5 % H_2O_2 in 10 ml 0.1 % acetic acid buffer (pH 5.0). Bound peroxidase was determined by adding 100 μl/well TMBZ substrate solution and incubating for 1 h at 25 ℃. The reaction was stopped by adding 50 μl 2 N H_2SO_4 per well. Absorbance was recorded at 450 nm with an automatic immuno-microplate reader NJ-2300 (Inter Med Japan, Tokyo, Japan). Each sample was analyzed three times. The cross-reactivities of KTM-205-POD and KTM-240-POD against DADON, % relative to TANIV, were 0.06 % and 100 %, respectively[12].

The results of ELISA were fitted to sigmoidal inhibition curves by plotting log toxin concentration (ng/ml) against relative absorbance (%B/B_0) (Fig. 3). Straight-line standard curves were generated by logit-log transformations[14] of the standard curves, and the unknown concentrations were interpolated from the linearized curves.

TLC Trichothecene standards (4-ANIV, NIV, 3-ADON, and DON) and sample solutions were spotted on silica gel 70 pre-coated plates (Wako Pure Chemical Ind., Osaka, Japan) and developed in chloroform:methanol (9:1, v/v), sprayed with 20 % (w/v) aluminum chloride solution ($AlCl_3 \cdot 6H_2O$) in ethanol:water (50:50, v/v) and baked at 110℃ for 10 min. Trichothecene standards were visualized as blue fluorescent spots under UV light (365 nm).

GC-MS NIV and DON compounds were derivatized by mixing 40 μl of the sample solution with 200 ng internal standard (scirpenetriol). The mixture was evaporated to dryness, dissolved in 25 μl TMS reagent, and allowed to stand for 10 min at 50 ℃ in a closed glass vial (1 ml vol.). The solution was diluted with 400 μl *n*-hexane, added with 400 μl phosphate buffer (0.13 M, pH 1.0) to decompose unreacted reagent, and vortex-mixed until the *n*-hexane layer became transparent. An aliquot (2 μl, equivalent to 0.05 mg of the original culture) of the resulting *n*-hexane layer was injected into the column.

For GC-MS, a Shimadzu GCMS-QP 5000 instrument (Shimadzu Co., Kyoto, Japan) was used, with a selected ion monitoring mode under the following conditions: 30 m x 0.25 mm i.d. DB-1 fused silica capillary column (J&W, CA, USA); carrier gas He at a flow rate of 2.0 ml/min; column temperature held for 5 min at 120 ℃ and then increased to 280 ℃ at 8 ℃/min; injector temperature of 280 ℃; interface temperature of 250 ℃; 70 eV ionizing voltage; 300 μA ionizing current; 1 scan/s

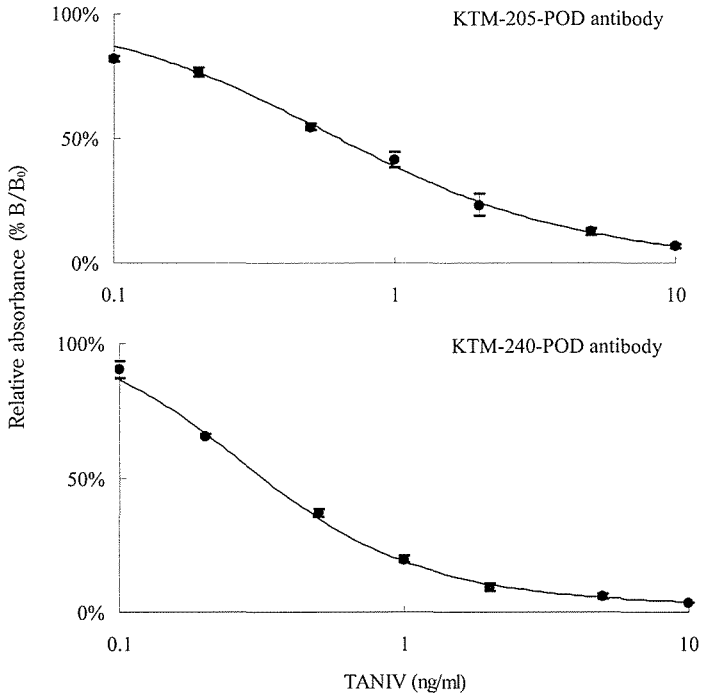


Fig. 3. Standard curves of ELISA for two antibodies, with TANIV concentrations ranging from 0.1 to 10 ng/ml. Toxin concentrations on the x-axis are in log scale. Each point represents the mean (± standard deviation) of three replicates.

scanning rate; and 5 points/s sampling rate for selected ion monitoring. Selected ions were m/z 422 and 407 for DON, 392 and 377 for 3-ADON, 407 and 392 for 15-ADON, 379 and 289 for NIV, 480 and 465 for 4-ANIV, and 379 and 283 for the internal standard. The detection limits for DON and NIV standards were about 0.5 and 0.25 ng, respectively. The recoveries of NIV and DON at a level of 1 mg/kg were 101 % and 100 %, respectively. The peak area ratio of toxin to the internal standard was compared by a calibration curve generated using different concentrations of DON, 3-ADON, 15-ADON, DADON, NIV and 4-ANIV standards.

Results and Discussions

Several ELISA methods were reported for individual detection of DON[15-17] and 3-ADON[18, 19], simultaneous detection of DON, 3-ADON and 15-ADON[20, 21], and DON and 3-ADON[22], and one attempt to develop ELISA for NIV[23]. In the present study, NIV, DON and their derivatives were partially acetylated to TANIV and DADON, respectively, before ELISA. The monoclonal antibodies used, KTM-205-POD and KTM-240-POD, were highly specific to TANIV and TANIV + DADON, respectively.

A total of 16 strains of *F. graminearum* isolated from wheat and barley grains were cultured on polished rice substrate, and the extracts were partially acetylated before ELISA. For initial screening of NIV production, KTM-205-POD antibody against TANIV was used. Twelve strains yielded high

concentrations of NIV and its derivatives which were determined as TANIV, ranging from 40 to 922 mg/kg. In contrast, four strains yielding concentrations below 17 mg/kg (strains codes 6, 10, 11 and 16) were considered non-producers of NIV (Table 1).

All partially acetylated extracts were also screened by ELISA using KTM-240-POD antibody and concentrations were determined as TANIV + DADON. Only one strain (strain code 16) that produced a concentration below 15 mg/kg was considered a non-producer. Positive samples ranged from 62 to 898 mg/kg (data not shown). However, three of the positive samples (strain code 6, 10 and 11) were negative (<17 mg/kg) when KTM-205-POD antibody was used, suggesting a production of DON-related trichothecenes with estimated concentrations ranging from 238 to 708 mg/kg (Table 1). Twelve samples were positive for TANIV and TANIV + DADON using the two antibodies and the concentrations obtained differed by 25 mg/kg, confirming the ability of these strains to produce NIV-related trichothecenes. The high variability of these concentrations may be attributed to the genetic diversity among the isolates of *F. graminearum*[24], hence, the ability of each isolate to produce different amounts of structurally-related compounds. One sample (strain code 16) was considered negative in both assays. All the ELISA results were confirmed by TLC analysis using 4-ANIV, NIV, DON and 3-ADON standards (Table 1).

Subsequently for quantitative confirmation, all the sample residues were derivatized as TMS ethers (Fig. 4) before GC-MS analysis. Twelve strains produced NIV (19-290 mg/kg) and 4-ANIV (18-

Table 1. Determination of total NIV- and DON-related trichothecenes, produced by single-spore cultures of *F. graminearum* on polished rice substrates, using TLC, GC-MS and ELISA methods.

Strain code	GC-MS (mg/kg)					ELISA[1] (mg/kg)	
	4-ANIV	NIV	DON	3-ADON	DADON	TANIV[2]	DADON[3]
1	56	32*	0	0	0	121	21
2	23	39*	0	0	0	109	20
3	26	65*	0	0	0	143	0
4	0	19*	0	0	0	40	22
5	64*	26*	0	0	0	144	0
6	0	0	218*	203*	7	17	708
7	164*	290*	0	0	0	922	0
8	36*	94*	0	0	0	261	0
9	24*	43*	0	0	0	111	9
10	0	0	135*	93*	0	1	317
11	0	0	109*	55*	1	1	238
12	18	54*	0	0	0	143	5
13	25*	49*	0	0	0	111	0
14	31*	52*	0	0	0	114	0
15	18	40*	0	0	0	99	7
16	0	5	0	0	0	10	5

[1] Total NIV- or DON-related trichothecenes were determined by ELISA after acetylation of *F. graminearum* extracts into TANIV and DADON, respectively.

[2] Concentrations obtained using KTM-205-POD.

[3] DADON concentrations estimated from those values obtained using KTM-240-POD minus KTM-205-POD, with negative results given as zero.

* TLC positive samples.

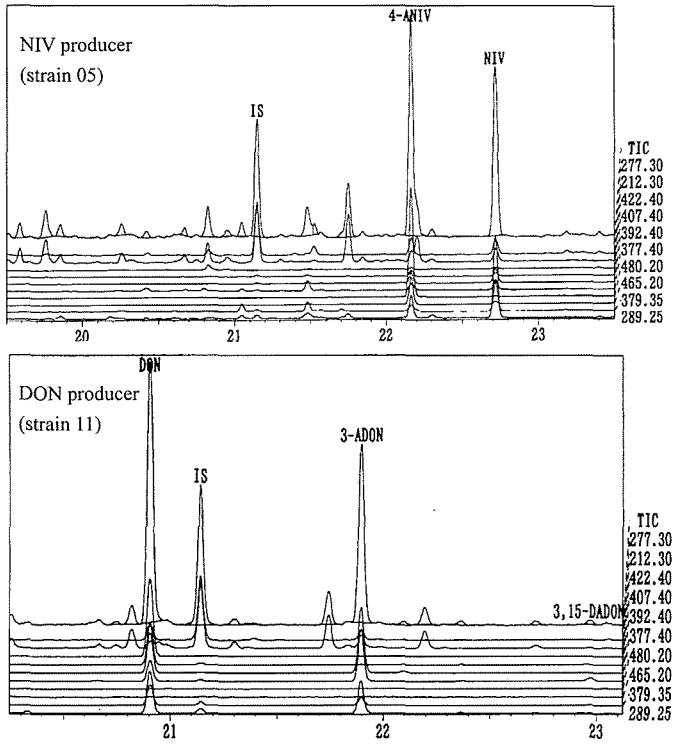


Fig. 4. GC-MS chromatograms of TMS-derivatized extracts of *F. graminearum* cultures.

164 mg/kg), 3 strains produced DON (108-217 mg/kg), 3-ADON (55-203 mg/kg) and 3,15-DADON (0.6-7.3 mg/kg), and one strain did not produce a significant quantity of either toxin (Table 1). In order to correlate ELISA and GC-MS results, the concentrations of TANIV and DADON levels determined by ELISA and those of NIV and 4-ANIV, and DON, 3-ADON and 3,15-DADON determined by GC-MS were expressed as NIV and DON equivalents, respectively. The results of ELISA and GC-MS for total NIV and DON showed a good correlation (r^2 = 0.9875; $p<0.001$) (Fig. 5). However, the results obtained by ELISA and GC-MS differed by 5 to 32 % for NIV- and 12 to 27 % for DON-positive samples, with ELISA values always higher than the GC-MS values (Table 2) as can be seen from the slope of the regression analysis of the data (Fig. 5).

Using TANIV, dissolved in PBST containing 10 % ethanol, as a standard the detection limit (80 % relative binding) was 0.16 and 0.13 ng/ml for KTM-205-POD and KTM-240-POD antibodies, respectively. However, in this study ELISA was used for the extracts of polished rice culture with minimal clean-up, and hence, can suffer from an overestimation of DON or NIV concentration due to minor components in the mixture that provide an opportunity for cross reactivity[25, 26]. A cut-off limit of 25 mg/kg is considered necessary to eliminate false positive results. Isolated strains of *F. graminearum* showed high variability in their toxin production. It is highly possible that several putative trichothecene precursors and shunt metabolites produced by *F. graminearum*[25, 27] which could not be detected by GC-MS without specific standards but were present in the culture extracts, cross-reacted in the ELISA technique and increased the overall response.

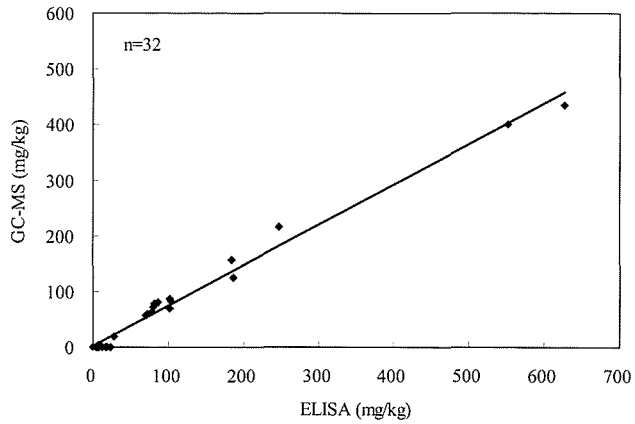


Fig. 5. Correlation of total NIV- and DON-related trichothecenes in *F. graminearum* extracts determined by ELISA and GC-MS. A linear regression equation of y=1.346+0.729x, with r^2=0.9875 ($p<0.001$) was obtained.

Table 2. ELISA and GC-MS quantification of total NIV- and DON-related trichothecenes produced on polished-rice substrates by *F. graminearum*.

Strain Code	NIV (mg/kg)		DON (mg/kg)	
	GC-MS[1]	ELISA[2]	GC-MS	ELISA
1	82	86	0	17
2	60	72	0	23
3	88	102	0	0
4	19	28	0	19
5	82	103	0	0
6	0	12	401	552
7	435	626	0	0
8	125	187	0	0
9	64	79	0	8
10	0	1	217	247
11	0	1	158	186
12	70	102	0	4
13	71	79	0	0
14	79	81	0	0
15	56	70	0	7
16	5	7	0	4
Mean[3]	95	125	259	331

[1] Concentrations of DON, 3-ADON and DADON, and NIV and 4-ANIV in *F. graminearum* extracts determined by GC-MS were expressed as DON and NIV equivalents, respectively.

[2] Concentrations of total NIV- and DON-related trichothecenes were determined by ELISA after acetylation of *F. graminearum* extracts (KTM-205 antibody for NIV and KTM-240 for simultaneous NIV and DON detection).

[3] Mean concentrations of positive samples.

Independent of the methodology used and according to a previous report[8], the isolates used in this study could be classified into two types: twelve isolates (80 %) were NIV-type strains producing also 4-ANIV and 3 (20 %) were DON-type strains producing also 3-ADON. ELISA assays using KTM-205-POD antibody would be useful for screening total NIV production, while KTM-240-POD would be applicable for general screening of total NIV and DON production. This could be achieved by converting DON, NIV and their derivatives to acetylated forms using the acetylation procedure described here. ELISA assay presented in this study requires minimal sample clean-up prior to assay, can analyze 12 samples per microtiter plate within approximately 24 h, and allows rapid processing of a large number of samples in batches. Thus, more than 100 samples can be assayed by ELISA in a day compared to a week for the same number of samples by GC-MS. The results of the present study show that ELISA has potential as a quick and simple method to differentiate NIV- and DON-producing *F. graminearum* strains that show high toxin production when cultured on polished rice substrate. However, samples suspected of having total levels of NIV- or DON-related trichothecenes below 25 mg/kg, must be analyzed by GC-MS to exclude false positive identification.

References

1) Nelson, P.E., Toussoun, T.A., Marasas, W.F.O.: *Fusarium* species: an illustrated manual for identification, (1983), Pennsylvania State University Press, University Park, USA
2) Snijder, C.H.A.: Neth. J. Plant Pathol., **96**, 187-198 (1990)
3) Koizumi, S., Kato, H., Yoshino, R., Komada, H., Ichinoe, M., Umehara, Y., Hayashi N.: Bull. Inst. Compr. Agr. Sci. Kinki Univ., **5**, 31-45 (1997)
4) Yoshizawa, T.: "Trichothecenes - chemical, biological and toxicological aspects" (ed. Ueno, Y.), pp. 195-209 (1983), Elsevier, Amsterdam
5) Yoshizawa, T.: Bull. Inst. Compr. Agr. Sci. Kinki Univ., **5**, 23-30 (1997)
6) Yoshizawa, T., Matsuura, Y., Tsuchiya, Y., Morooka, N., Kitani, K., Ichinoe, M., Kurata, H.: J. Food Hyg. Soc. Japan, **20**, 21-26 (1979)
7) Tanaka, T., Hasegawa, A., Yamamoto, S., Lee, U.-S., Suigura, Y., Ueno Y.: J. Agric. Food Chem., **36**, 979-983 (1988)
8) Mirocha, C.J., Abbas, H.K., Windels, C.E., Xie, W.: Appl. Environ. Microbiol., **55**, 1315-1316 (1989)
9) Yoshizawa, T., Jin, Y.-Z.: Mycotoxins, **47**, 15-18 (1998)
10) Snijder, C.H.A., Krechting, C.F.: Can. J. Botany., **70**, 1570-1576 (1992)
11) Ryu, J.C., Ohtsubo K., Izumiyama N., Nakamura K., Tanaka T., Yamamura H., Ueno Y.: Fund. Appl. Toxicol., **11**, 38-47 (1988)
12) Ikeda, K., Munakata, Y., Morita, K., Kusada, O., Yoshizawa, T., Kohno, H.: "New horizon of mycotoxicology for assuring food safety' (eds, Yoshizawa, T., Kumagai, S., Goto, T.) pp.233-239 (2004), Jpn Assoc. Mycotoxicol., Tokyo.
13) Yumbe-Guevara, B.E., Yoshizawa, T.: Mycotoxins, **53**, 87-93 (2003)
14) Rodbard, D., Bridson, W., Rayford, P.L.: J. Lab. Clin. Med., **74**, 770 (1969)
15) Casale, W.L., Pestka, J.J., Hart, L.P.: J. Agric. Food Chem., **36**, 663-668 (1998)
16) Mills, E.N.C., Alcock, S.M., Lee, H.A., Morgan, M.A.: Food Agric. Immunol., **2**, 109-118 (1990)

17) Xu, Y.C., Zhang, G.S., Chu, F.S.: J. Assoc. Off. Anal Chem., **71**, 945-949 (1988)
18) Kemp, H.A., Mills, E.N.C., Morgan, M.R.A.: J. Sci. Food Agric., **37**, 888-894 (1986)
19) Wang, N.A., Kierek-Jaszczuk, D., Marquardt, R.R., Frohlich, A.A., Abramson, D.: J. Food Prot., **59**, 525-533 (1996)
20) Usleber, E., Märtlbauer, E., Dietrich, R., Terplan, G.: J. Agric. Food Chem., **39**, 2091-2095 (1991)
21) Maurice, N., Laurel, D., Miles, C.O., Jones, W.T.: Food Agric. Immunol., **5**, 199-209 (1993)
22) Maragos, C.M., McCormick, S.P.: Food Agric. Immunol., **12**, 181-192 (2000)
23) Ikebuchi, H., Teshima, R., Hirai, K., Sato, M., Ichinoe M., Terao, T.: Biol. Chem. Hoppe-Seyler, **371**, 31-36 (1990)
24) Dusabenyagasani, M., Dostaler, D., Hamelin, R.C.: Can. J. Plant Pathol., **21**, 308-314 (1999)
25) Abouzied, M.M., Azcona, J.I., Braselton, W.E., Pestka, J.J.: Appl. Environ. Microbiol., **57**, 672-677 (1991)
26) Park, J.J., Chu, F.S.: J. AOAC Int., **79**, 465-471 (1996)
27) Sinha, R.C., Savard, M.E.: Can. J. Plant Pathol., **18**, 233-236 (1996)

ニバレノール及びデオキシニバレノール産生性 *Fusarium* spp. の選抜への酵素免疫測定法の応用

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ニバレノール（NIV）とデオキシニバレノール（DON）の部分アセチル体，すなわち 3,4,15-triacetyl-NIV（TANIV）及び 3,15-diacetyl-DON（DADON）に対して高い特異性を示す 2 種類のモノクローナル抗体（KTM-205-POD, KTM-240-POD）を用いて，*Fusarium* spp. の NIV 及び DON 産生能を検討した．16 菌株の *F. graminearum* を白米培地で培養し，培養抽出液中の NIV，DON 及び関連誘導体を部分アセチル化して TANIV もしくは DADON に誘導化してから，競合的直接 ELISA 法により定量した．また，GC-MS により培養物中のトキシン濃度を定量した．ELISA と GC-MS による定量値の相関は $r^2 = 0.9875$ ($p<0.001$) であり，本 ELISA（カットオフ値，25 mg/kg）が NIV 及び DON 産生菌の迅速かつ簡便な選抜法として適用できることが示された．

キーワード：ELISA，ニバレノール，デオキシニバレノール，*Fusarium graminearum*