Evaluation of toxicities of 7-hydroxyisotrichodermin and 8-hydroxyisotrichodermin, shunt intermediates in the biosynthetic grid of deoxynivalenol, by using a sensitive yeast assay

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Abstract

_Fusarium graminearum_ causes a disease of wheat and barley known as _Fusarium_ head blight. It contaminates the grains with trichothecene mycotoxins such as deoxynivalenol (DON). As shunt intermediates in the DON biosynthetic pathway, 7-hydroxyisotrichodermin (7-HIT) and 8-hydroxyisotrichodermin (8-HIT) are known. However, their activities have not been previously evaluated. In this study, we performed toxicity assays of these trichotheccenes by using a sensitive yeast bioassay that we have recently established. The IC50 of 7-HIT and 8-HIT were in the range of 20-40 µg/ml, while the IC50 of DON was approximately 1.5 µg/ml. Although the toxicity of these shunt metabolites remains to be investigated in animal systems, our present data indicate that 7-HIT and 8-HIT may not be major issues that require regulation in agricultural products.

Keywords
deoxynivalenol; _Fusarium_; minor intermediates; shunt pathway

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Fusarium head blight (FHB) is a serious disease of wheat and barley caused mainly by _Fusarium graminearum_ and _Fusarium culmorum_. The infected grains are often contaminated with trichotheccenes such as deoxynivalenol (DON; Fig. 1), nivalenol (NIV), and acetylated derivatives thereof1,2,3. These mycotoxins, which are characterized by the presence of a keto-group at C-8, are collectively termed type B trichotheccenes. The toxicities of these mycotoxins are significantly affected by the side-chain oxygenation and acetylation patterns4,5. With regard to DON, a provisional limit (1.1 ppm in wheat in Japan) has been set in many countries to ensure the safety of cereal grains and processed products.

DON biosynthesis starts with cyclization of all-trans-farnesyl pyrophosphate to give trichodiene, followed by four oxygenation steps and a second non-enzymatic cyclization yielding isotrichodermol. As major intermediates of the DON pathway, isotrichodermol, isotrichodermin, 15-deacetylcalonectrin, calonectrin, and 3,15-diacetyldeoxynivalenol are known6,7. The toxicities of these major intermediates were previously evaluated using either animal or plant systems8. However, the toxicities of shunt intermediates of DON, such as 7-hydroxyisotrichodermin (7-HIT) and 8-hydroxyisotrichodermin (8-HIT) (see Fig. 1) have not been examined so far.

Previously, we developed a sensitive yeast bioassay for trichotheccenes by utilizing a gene deletion mutant of three resistance genes against trichotheccenes: _pdr5_, _erg6_, and _rpbl_ in _Saccharomyces cerevisiae_ BY47429.

Fig. 1 Chemical structures of deoxynivalenol (DON), 7-hydroxyisotrichodermin (7-HIT), and 8-hydroxyisotrichodermin (8-HIT).
Triple null mutant pdr5Δ erg6Δ rpb4Δ cells showed a high sensitivity to various trichothecenes; thus, in order to clarify the toxicity of 7-HIT and 8-HIT, we determined their IC50 by using this recently established yeast bioassay.

DON, 7-HIT, and 8-HIT were prepared from F. graminearum culture as described previously[8,10]. A standard solution of DON (100 µg/ml in acetonitrile; Wako Pure Chemical Industries, Ltd., Osaka) was used as a standard sample of DON with a known concentration. With regard to 7-HIT and 8-HIT, the weight of purified samples was quantified using quantitative 1H NMR (qNMR) analysis with JNM-ECX 500 (JEOL Ltd., Tokyo) spectrometer operating at 500.16 MHz. Briefly, the samples were dissolved in CDCl3-d (Sigma-Aldrich, St. Louis, MO), and 1,4-bis(trimethylsilyl)benzene-d4 (1,4-BTMBS-d4, Wako Chemical) was used as an internal calibrant. Fourier transformation of the free induction decay was carried out without processing of zero filling and an exponential window function by using Delta software (ver. 4. 3. 6; JEOL Inc., Peabody, MA). During data processing, phase and baseline corrections were done manually, and the signals were also integrated manually. The weight of the analyte was calculated from the obtained spectrum as previously described[11].

The concentrations of purified DON, 7-HIT, and 8-HIT used for the assay were determined on the basis of a calibration curve considering the relationship of concentrations and peak areas of these trichothecenes separated by an HPLC system (JASCO Co., Model LC-2000 plus, Tokyo). For determination of peak areas, aliquots of standard trichothecene solutions were injected onto an ODS column (Pegasil ODS SP100 4.6 × 250 mm, Senshu Scientific Co., Ltd., Tokyo). HPLC conditions were as follows: DON was eluted with isocratic elution of 20% of acetonitrile (flow rate: 1 ml/min), and monitored by UV absorption at 254 nm, while 7-HIT and 8-HIT were eluted with a linear gradient of 20-100% acetonitrile (flow rate: 1 ml/min) and monitored at 195 nm. The purity of each trichothecene was confirmed to be >97%.

The toxicity of trichothecenes was evaluated by measuring OD620 of a toxin hyper-sensitive pdr5Δ erg6Δ rpb4Δ triple deletion mutant of S. cerevisiae BY4742 grown in 96-well titer plates, following a previously published protocol with a minor modification[9]; the concentration of SDS in the assay medium was reduced to 0.003% to ensure dense growth in the absence of trichothecenes while keeping sufficiently sparse growth in their presence. The final concentration of each toxin added to the cell culture was 0, 0.5, 1.25, 2.5, 5.0, 12.5, 25, or 50 µg/ml. DMSO was used as a vehicle and the final concentration of DMSO was 2%.

As shown in Fig. 2, the IC50 of 7-HIT and 8-HIT was in the range of 20–40 µg/ml, whereas that of DON was approximately 1.5 µg/ml. The results suggest that while these minor metabolites are toxic, their inhibitory action on yeast growth is an order of magnitude lower than that of DON. Although the toxicities of these shunt metabolites remains to be investigated in animal systems, the comparative growth inhibition data obtained in the yeast assay appear to be generalizable to other organisms. For example, the values of IC50, where T-2 toxin (0.0015 µg/ml) < 4-acetyldeoxinivalenol (0.1 µg/ml) < DON (1.5 µg/ml) < NIV (7 µg/ml) < 3-acetyldeoxinivalenol (50 µg/ml), obtained using the yeast assay[9] are also conserved in Vero cell lines, in which the toxicities as evaluated by 50% PSI (nmol to require 50% protein synthesis inhibition in the reaction mixture), are T-2 toxin (14 nmol), < 4-acetyldeoxinivalenol (288 nmol) < DON (1499 nmol) < NIV (8131 nmol) < 3-acetyldeoxinivalenol (26,279 nmol)[12]. The low toxicities of 7-HIT and 8-HIT may presumably be attributed to the existence of an acetyl group at C-3, which reduces trichothecene-induced toxicities dramatically[13].

Together with our current knowledge that trichothecene precursors are barely detectable in agricultural products[14], the low toxicities of 7-HIT and 8-HIT appear not to support the necessity of their regulation in agricultural products.

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